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Review Article

Which animal models should be used in the search for new antiepileptic drugs? A proposal based on experimental and clinical considerations

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OBJECTIVES IN THE SEARCH FOR NEW ANTIEPILEPTIC DRUGS

It has been estimated that about 20% of patients with epilepsy cannot be treated successfully with current antiepileptic drugs¹⁴. It should be emphasized, however, that this global figure represents the average drug resistance of patients with diverse types of epilepsy. Considered individually, the prognoses for the various types of epilepsy differ considerably. A large number of epileptic syndromes of childhood and adolescence cannot be successfully treated with current antiepileptic drugs, e.g., West syndrome, early myoclonic encephalopathy, Lennox-Gastaut syndrome, epilepsy with myoclonic astatic seizures, epilepsy with myoclonic absences, and severe myoclonic epilepsy in infancy¹⁵. In adults, the highest drug resistance of about 70% is found in patients with complex partial seizures¹⁶, the most common type of epilepsy (see Table I), whereas the prognosis of most types of primary generalized seizure types is much better. Thus, there is a need for new antiepileptic drugs which should be more effective than existing drugs in intractable epilepsies, such as those with complex partial seizures. In addition, there is growing concern about the acute and chronic toxicity of currently used antiepileptic drugs¹⁷. For instance, the favourable clinical efficacy of valproic acid in epileptic syndromes of childhood and adolescence is severely impaired by the potential embryotoxicity and hepatotoxicity of this drug¹⁸. Thus, newly developed drugs should be less toxic than existing drugs. Drug toxicity testing is part of the preclinical evaluation of any newly developed compound, whereas there is no preclinical efficacy testing against resistant seizure types, which is due to the lack of suitable animal models. The various animal models for epilepsy that are currently used in the search for new anticonvulsant drugs are models in which traditional antiepileptic drugs are active, which may hamper the identification of potentially useful compounds with new mechanism of action. Thus there is a need for experimental models of intractable epilepsy in order to study mechanisms of drug resistance and to eventually discover new drugs that are effective in patients not controlled

on current antiepileptic medication. Several models which may be suitable in this respect have recently been proposed¹⁹. The Antiepileptic Drug Development Program of the National Institute of Neurological and Communicative Disorders and Stroke (NINCDS) of the National Institutes of Health (NIH) U.S.A., which since its initiation in 1975 has stimulated the development of various new antiepileptic drugs, is primarily based on only 2 seizure models, the maximal electroshock seizure (MES) test and the subcutaneous (s.c.) pentylenetetrazol (PTZ) test^{20,21}. The MES test is thought to predict drugs effective against generalized seizures of the tonic-clonic (grand mal) type, whereas the s.c. PTZ test is used to find drugs effective against generalized seizures of the petit mal (absence) type²⁰. However, most patients with these seizure types can be successfully treated with existing antiepileptic drugs (see Table I), while there is a much more pressing clinical demand to find new drugs for the treatment of other, more difficult to control seizure types and epilepsy syndromes, such as complex partial seizures, West syndrome and Lennox-Gastaut syndrome. Consequently, although the predictive value of the MES and PTZ models is still of use for antiepileptic drug development, models of other types of epilepsy should be added to these traditional models early during drug evaluation. In this review we will (1) critically discuss the advantages and disadvantages of the diverse animal models which are available, (2) propose models which should be used in a standardized way in the search for clinically relevant new antiepileptic drugs, (3) outline the efficacy of clinically established antiepileptic drugs in these models, and (4) compare the efficacy of new anticonvulsant drugs in these models with initial clinical data on these compounds. With respect to the diverse models discussed, it should be noticed that the term 'petit mal' will be used to comprise both absence and myoclonic seizures. The differences between these seizure types in regard to antiepileptic drug sensitivity and the consequences for the predictive value of so-called petit mal models will be discussed in the last section of this review. It should be stressed already here, however, that in terms of antiepileptic drug efficacy the petit mal models described in this review are

TABLE I
Spectrum
Signs and
enriched to
Drug

Phenytoin
Carbamazepine
Phenobarbital
Primidone
Valproic acid
Ethosuximide
Benzodiazepines

Relative frequency (%)
of seizure types

* Develop
** From G:

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TABLE I

Spectrum of clinical activity of common antiepileptic drugs

Signs and abbreviations: +, ++, +++, antiepileptic efficacy; NE, not effective; GTCS, generalized tonic-clonic seizures; GTS, generalized tonic seizures; GCS, generalized clonic seizures. For reference see Schmidt¹⁴.

Drug	Partial (focal) seizures		Partial seizures evolving to gen. seizures (GTCS, GTS or GCS)	Generalized seizures				
	Simple partial seizures	Complex partial seizures		Tonic and/or clonic (grand mal) seizures	Absence (petit mal) seizures	Myoclonic seizures (impulsive petit mal)	Lennox-Gastaut syndrome (myoclonic astatic epilepsy)	West syndrome (infantile spasms)
Phenytoin	+++	+++	+++	+++	NE	NE	NE	NE
Carbamazepine	+++	+++	+++	+++	NE	NE	NE	NE
Phenobarbital	+++	+++	+++	+++	NE	++	NE	NE
Primidone	+++	+++	+++	+++	NE	++	NE	NE
Valproic acid	+	+	+	+++	+++	+++	++	+
Ethosuximide	NE	NE	NE	NE	+++	+++	NE	NE
Benzodiazepines	++	++	++	++	+	+	++	+
				(Status +++)				
Relative frequency (%) of seizure type**	10	30-40	10	10-40	5-10	4	1-6	1-2

* Development of tolerance.

** From Gastaut et al.⁵⁸ and Janz⁵²; based on data from 12500 patients with epilepsy.

models of myoclonic seizures but not models of absence seizures.

ANIMAL MODELS FOR THE SEARCH OF NEW ANTI-EPILEPTIC DRUGS: MODELS OF SEIZURE STATES VS. MODELS OF EPILEPSY

The innumerable animal models that are used in epilepsy research have been the subject of one volume¹³⁶ and several reviews^{5-8, 50, 64, 77, 89, 111, 162}. Only models potentially useful for antiepileptic drug development will be shortly discussed here (see Table II). Experimental research on epilepsy and antiepileptic drugs has mostly been done in mice and rats in which seizures were induced by chemical or electrical means. Although these rodent models, such as the MES and PTZ models, have proved useful for the identification of drugs with anticonvulsant action, they are obviously not closely related to human epilepsy but represent models for induction of single epileptic seizures

rather than models of epilepsy. An ideal model of epilepsy should show the following characteristics: (1) the development of spontaneously occurring recurrent seizures, (2) a type of seizure similar in its clinical phenomenology to seizures occurring in human epilepsy, (3) an age-dependent onset of epilepsy similar to that observed in generalized epileptic syndromes in man, (4) the clinical seizures should be associated with epileptic-like activity in the EEG, (5) pharmacokinetics of antiepileptic drugs similar to those in humans thus allowing the maintenance of effective drug levels during chronic treatment, and (6) effective plasma concentrations of antiepileptic drugs similar to those required for control of the respective seizure type in humans. No model at present meets all these criteria. However, there are several more recent so-called genetic animal models of epilepsy, i.e., species or strains of animals with 'inborn' epilepsy, which resemble idiopathic epilepsy in humans more closely than any other experimental model.

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TABLE II

Experimental models of epilepsy

This table only summarizes the most important models that are currently used. For additional models see Purpura et al.¹³⁶. Abbreviations: β -CCM, methyl- β -carboline-3-carboxylate; DMCM, methyl-6,7-dimethoxy-4-ethyl- β -carboline-3-carboxylate; FG 7142, *N*-methyl- β -carboline-3-carboxamide; NMDA, *N*-methyl-D-aspartate; DDT, dichlorodiphenyltrichloroethane.

Models	References
(1) <i>Genetic animal models</i>	77,89
(a) With spontaneous recurrent seizures	
Epileptic dogs (focal seizures, generalized tonic-clonic seizures)	77,84,97
Rats with petit mal epilepsy	3,63,179,181
Tottering mice (focal seizures, petit mal seizures)	46,77,124,125
AE mice (tonic-clonic seizures)	92
C57BL/10B ^g mice (generalized seizures)	154
Quaking mice (spontaneous and reflex myoclonic and generalized tonic-clonic seizures)	154,157,169
B10 66.93 mutant hamsters (spontaneous and reflex myoclonic and generalized tonic seizures)	191
(b) With reflex seizures	
Baboons with photomyoclonic seizures	60,61,77,89,123
Photosensitive fowl (generalized tonic-clonic seizures)	20,53,77
Audogenic seizure susceptible mice and rats (running fits, generalized tonic-clonic seizures)	12,13,77,153
Gerbils (facial myoclonic and generalized myoclonic and tonic-clonic seizures in response to handling, change in environment or air blast)	35,77,88
El mice (limbic and generalized seizures in response to vestibular stimulation)	154
(2) <i>Electrically induced seizures</i>	164-166,168
(a) Threshold models (minimal and maximal electroshock seizure threshold)	165
(b) Maximal electroshock seizure (MES) test (generalized tonic seizures)	165
(c) Kindled (focal and secondarily gen.) seizures induced by repeated stimulation of various brain regions, the amygdala being the most sensitive structure	41,105,138
(3) <i>Chemically induced seizures</i>	
(a) Chemoconvulsants inducing generalized seizures after systemic administration	161
Pentylenetetrazol (gen., clonic and (in higher doses) tonic seizures)	164,166,168
Other GABA antagonists (e.g., bicuculline, picrotoxin, convulsant barbiturates, "cage convulsants," penicillin, lidocaine)	86,108,110
Inhibitors of GABA synthesis (inhibitors of pyridoxalphosphate, such as methylypyridoxine, l-isoleucine, thiosemicarbazide and D-penicillamine; competitive antagonists of GAD, such as 3-mercaptopropionic acid; and non-competitive GAD inhibitors, such as allylglycine)	86,108,110
Inverse benzodiazepine receptor agonists (e.g., β -CCM, DMCM, FG 7142)	129,160
Glycine antagonists (strychnine)	110
Cholinomimetic drugs (e.g., pilocarpine)	161,176
Excitatory amino acid receptor agonists (e.g., NMDA and kainic acid)	84,110
Miscellaneous convulsants (e.g., gamma-hydroxybutyric acid (petit mal seizures), DDT (myoclonus), methionine sulfoximine, fluroethyl)	158,161,180
(b) Chemoconvulsants used to induce focal seizures (after central administration), e.g., penicillin, kainic acid, quinolinic acid, pentylenetetrazol, ouabain	5,134,182
(4) <i>Focal seizures induced by (topical convulsant) metals applied to cortical areas:</i> e.g., aluminum cream model and cobalt model	18,187
(5) <i>Neurophysiological seizure models using recording from single neurons in intact animals, isolated tissue preparations or tissue cultures</i>	19,23,40,101,106

Genetic animal models of epilepsy

The advantages and drawbacks of these models have been reviewed recently⁷⁷. Despite the specific potential of genetic animal models for the development of new antiepileptic drugs, they are only rarely used in preclinical drug testing. As shown in Table II, genetic animal models can be subdivided into animals with spontaneously occurring recurrent seizures, and models in which seizures are induced by specific sensory stimulation in genetically susceptible animals, such as baboons, DBA/2 mice and gerbils. By definition of epilepsy, animals with chronically recurring, spontaneous seizures represent ideal models for human epilepsy; however, the disadvantage of such models for drug evaluation is that in most of the animals the naturally occurring seizures cannot be elicited at

will by an investigator, which makes drug efficacy studies time-consuming, especially when the seizure frequency is low. Among the animal species with spontaneously occurring seizures shown in Table II, epileptic dogs have proved useful as a model of focal and primary and/or secondary generalized tonic-clonic seizures^{77,84,97}. Besides idiopathic epilepsy, there occurs also symptomatic epilepsy in dogs, mostly due to brain tumours⁷⁷. It should be noted in this respect that no selectively bred dogs have been used in our studies on this species but the respective animals were patients of the Department of Small Animal Diseases in Berlin. The prevalence of epilepsy in the dogs seen at this clinic is 0.6%⁷⁷. Breed- and sex-related differences in prevalence and the age-dependent development of epilepsy in dogs have been described

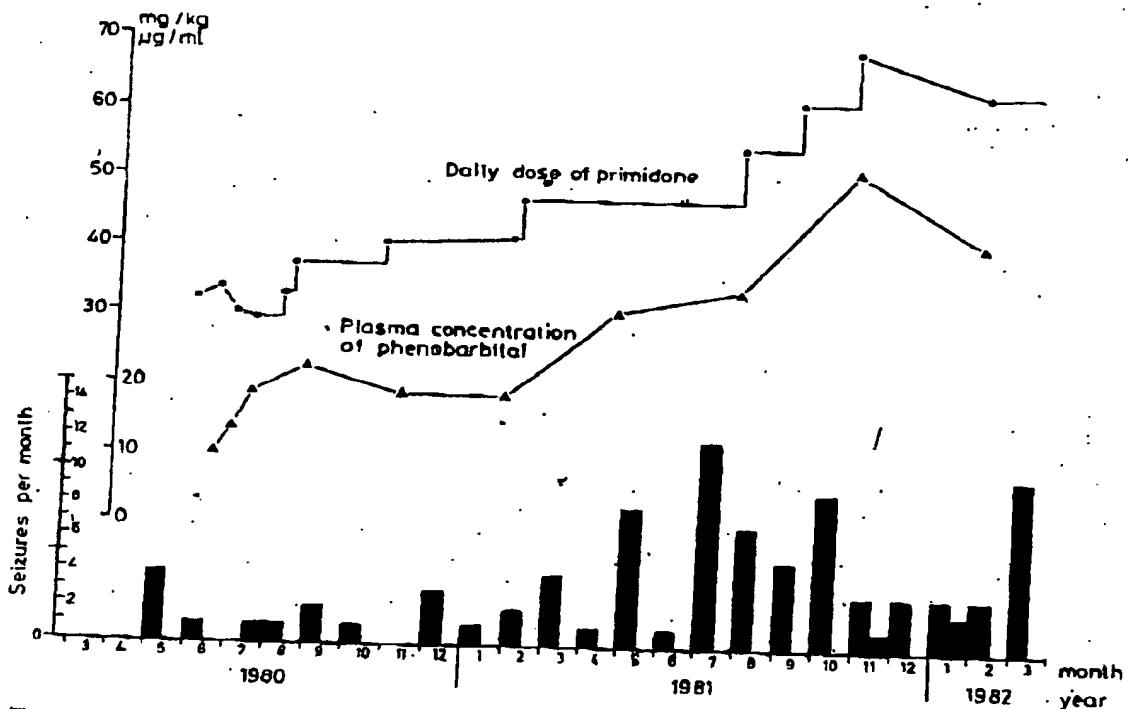


Fig. 1. Seizure frequency of an epileptic dog with generalized tonic-clonic seizures before and during continuous treatment with primidone. Age of onset of epilepsy in this male German shepherd was 11 months. Treatment with primidone was started 1 month thereafter, and phenobarbital plasma concentrations were monitored during primidone medication. Frequency of seizures is indicated by the number of generalized tonic-clonic seizures per month. Despite the continuous increase in primidone dosage, seizure frequency was not reduced in this drug-resistant dog (in other dogs with this type of seizures, daily oral doses of about 20–70 mg/kg were effective). Data are from D. Schwartz-Porsche (cited in Lüscher⁸⁴).

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elsewhere⁹⁷. Studies with commonly used antiepileptic drugs in epileptic dogs with generalized tonic-clonic seizures showed that 20–40% of the animals are refractory to high-dose antiepileptic drug therapy^{94,97}. Focal seizures were more difficult to treat than generalized tonic-clonic seizures⁹⁴, which is consistent with clinical experience in human epilepsy¹⁴⁶. An example of a drug-resistant dog is shown in Fig. 1. These animals represent an ideal source for the study of causative factors in intractable epilepsy and for the search of new antiepileptic drugs with efficacy against seizures that did not respond to previous therapy. However, the epileptic dog model is not suited for drug screening purposes since this is a model for chronic epilepsy and, consequently, drug-efficacy studies are time-consuming. Furthermore, there are logistical difficulties in obtaining an adequately high number of epileptic dogs for long-term studies. Although selectively bred epileptic beagles have been used for drug evaluation⁹⁷, due to the low seizure frequency, seizures in these animals had to be induced by electroshock or PTZ to allow acute drug efficacy studies. *Rats with spontaneously occurring petit mal epilepsy and tottering mice* have advantages in this respect since the seizure frequency in these animals is so high that acute drug efficacy studies can be carried out without 'unnatural' seizure induction⁷⁷. Approximately 15–30% of Sprague-Dawley and Wistar rats exhibit spontaneously occurring spike-wave discharges (7–11/sec) which are associated with petit mal-like behavioural seizure components, such as behavioural arrest and myoclonic twitching, mostly limited to the facial musculature^{3,6,179,181}. The spontaneous bursts last from 0.5 to 40 sec and occur hundreds of times a day. Age is a critical factor in the development of the EEG paroxysms; usually no epileptic EEG signs are observed in male and female animals before 14–18 weeks. Once developed, the epileptic discharges seem to persist for the whole life-time. The incidence of seizures can be markedly increased when pairs of epileptic rats are used for breeding, thus demonstrating the genetic transmission of the phenomenon. The pharmacological sensitivity of the seizures is similar to that of human absence seizures, except that phenobarbital is active at low doses (see Table V). The available

data thus indicate that these rats are a suitable model of petit mal epilepsy. Since this is a chronic model, the animals can easily be used for chronic drug efficacy studies. The rat petit mal model has thus several advantages in comparison to most other models, including the PTZ test, which are used for this seizure type. The homozygous tottering mouse originally described by Green and Sidman⁹⁶ is a presumed single-locus mutant, phenotypically characterized by spontaneous epileptic seizures¹²⁵. By 3–4 weeks of age, affected homozygotes can be recognized by a broad-based, ataxic gait. Spontaneous focal motor seizures (unilateral clonic jerks of the limbs with secondary generalization) are observed 1–3 days later and occur one or more times per day throughout the normal life span. In studies on tottering mice in our laboratory, the focal motor seizures occurred irregularly a few times a day and 93% of the seizures lasted for 15 min or longer (D. Schultz, cited in ref. 77). Studies on the pharmacological sensitivity of the focal seizures in tottering mice showed that the seizures are not affected by ethosuximide and valproic acid but are potently suppressed by diazepam⁷⁷. A second distinct seizure pattern in homozygous tottering mice are spike-wave petit mal seizures. As early as 32 days postnatal, bilaterally synchronous 6–7/sec, spike-wave discharges appear as spontaneous bursts in EEG recordings¹²⁶. These spike-wave bursts, 0.3–10 sec in duration, occur hundreds of times per day and are in each case accompanied by a behavioural petit mal seizure, which is very similar to seizures observed in the rats described above. These petit mal seizures are present at least through 10 months of age. As shown in Table V, the petit mal seizures are blocked by ethosuximide, diazepam and phenobarbital, whereas phenytoin is not effective⁴⁹. The main disadvantage of tottering mice is the problem of getting enough epileptic homozygotes for pharmacological studies. At least in our laboratory, breeding with homozygous mice proved to be impossible and heterozygous animals had to be used to get homozygotes with seizures for the experiments⁷⁷. This procedure is very time-consuming and limits the potential of tottering mice as model for anticonvulsant drug evaluation.

Spontaneously occurring generalized tonic-

clonic seizures, an interesting to occur for routine interestinously a mal species responsive responsive seizure-non-er efficac MES and 'normal' worthy of ation of a mals may fulness. In the C57B leptic sei strains ha as to esti drug eval which, like recessive homozygous and/or eli clonic sei pomoyelin epileptic c have been macologic curring se handling-phenobarl valproic a pam were gested fr prove to t reliable an trial new as cal motor experimen value of th Anothe have been hamsters¹⁹. hamsters,

clonic seizures have been described recently in *AE mice*, an inbred mouse strain with interesting features for epilepsy research⁹¹. However, seizures occur too infrequently in these mice to be of use for routine antiepileptic drug efficacy studies. An interesting observation of *AE* mice, which has previously also been reported in other epileptic animal species^{e.g.}²⁷, is that the animals are more responsive to electrical seizure induction but less responsive to anticonvulsants as compared with seizure-non-susceptible strains of animals⁹². The lower efficacy of major antiepileptic drugs against MES and PTZ seizures in *AE* mice compared to 'normal' mice is an interesting phenomenon worthy of further studies and indicates that evaluation of anticonvulsant drugs in non-epileptic animals may exaggerate their potential clinical usefulness. There are also other mouse strains, e.g., the *C57BL/10Bg* strain, in which spontaneous epileptic seizures occur¹³⁴, however, none of these strains has been pharmacologically characterized as to estimate its potential use for antiepileptic drug evaluation. An exception are *quaking* mice which, like the *tottering* mice, have an autosomal recessive genetic disorder¹⁵⁷. Animals being homozygous for this mutation exhibit spontaneous and/or elicited myoclonic and generalized tonic-clonic seizures which seem to be related to the hypomyelination disorder of these mice¹⁵⁴. For antiepileptic drug evaluation, seizures in *quaking* mice have been induced by handling, whereas the pharmacological sensitivity of the spontaneously occurring seizures has not been studied yet¹⁶⁹. The handling-induced seizures could be blocked by phenobarbital, phenytoin, carbamazepine and alproic acid, whereas ethosuximide and diazepam were relatively ineffective¹⁶⁹. It was suggested from these data that *quaking* mice may prove to be an inexpensive, simple, sensitive and reliable animal model for the assessment of potential new anticonvulsant drugs effective against focal motor seizures in humans¹⁶⁹, however, further experiments are necessary to prove the predictive value of this model.

Another species in which spontaneous seizures have been described to occur, are Syrian golden hamsters¹⁹¹. In the inbred line BIO 86.93 of Syrian hamsters, myoclonic and generalized tonic sei-

zures occur both spontaneously (J.E. Fisher, unpublished observations) and in response to mild stress¹⁹. Seizures occur in definable stages of hyperkinetic-ataxic gait, straub tail, falling, tonic hind limb extension, wading pool crawl, stereotyped head and psychomotor movements, followed by a paralytic state of extended rigid hind limbs with myoclonus of forelimbs and jaws. These symptoms may last for several hours. This clinical phenomenology of the seizure symptoms does not correspond to a seizure type in human epilepsy which limits the predictive value of the model. Phenytoin, phenobarbital, ethosuximide and trimethadione are not capable of blocking the seizures (phenytoin rather acts as a proconvulsant) while benzodiazepines are effective²⁰. Unfortunately, this interesting model for drug-resistant epilepsy is not generally available yet.

To summarize, with the exception of rats with spike-wave petit mal seizures, none of the described species with spontaneously occurring epileptic seizures is useful for acute antiepileptic drug efficacy studies, mainly because of logistical problems, such as availability of the animals and/or too low or irregular seizure frequency of the spontaneous seizures. However, it should be emphasized that animals with chronic epilepsy, such as epileptic dogs and rats, are ideal models for chronic drug efficacy studies and may thus be interesting for further evaluation of a new drug once this drug has been shown to be a valuable candidate for further development. In some species, i.e., quaking mice and mutant hamsters, seizures can also be induced by sensory stimulation, which may be an advantage for drug screening, but definite conclusions should await additional pharmacological characterization of these models.

As shown in Table II, there are several genetically predisposed animal species in which seizures do not occur spontaneously but can be induced by specific sensory stimulation, such as audiogenic or photic stimuli¹⁷. The major drawback of all these genetic animal models with 'reflex' seizures is that epilepsies characterized by specific modes of seizure precipitation, so-called reflex epilepsies, are rare in humans. Only about 5% of patients with epilepsy get focal or generalized seizures in response to sensory stimulation¹⁴. About one-third

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of these patients with reflex epilepsy responds to photic stimulation. Seizures in photosensitive epilepsy are mostly of the absence type or generalized tonic-clonic type, whereas complex partial or simple partial seizures are less frequent. Furthermore, it should be noted that photomyoclonic seizures can occur in non-epileptic patients, which means that animals (e.g., baboons) with photomyoclonic seizures are not necessarily models for epilepsy. The main advantage of genetic animal models with reflex seizures for anticonvulsant drug evaluation is that seizures can be easily and reproducibly evoked in these models without electrical or chemical means, and that the seizure types, at least in part, are similar in their clinical phenomenology to seizures occurring in human epilepsy.

A photomyoclonic syndrome in the baboon *Papio papio* was first reported by Killam et al. in 1966 (ref. 61). Since then, much work has been carried out on elucidating the characteristics of this syndrome^{60,123}. Myoclonic responses to intermittent photic stimulation occur in 60–80% of adolescent baboons *P. papio* from the Casamance region of Senegal, whereas seizure frequency is lower in *P. papio* from other regions. A minority of the animals (about 20%) show full tonic-clonic seizures upon stimulation. The photomyoclonic response of *P. papio* has been suggested as a model for photomyoclonic seizures and myoclonic petit mal epilepsy in humans^{60,123}. However, as shown in Table V, the sensitivity of this response to antiepileptic drugs is only in part similar to the human syndromes. Consistent with clinical experience, valproic acid, phenobarbital and benzodiazepines give complete protection against the myoclonic response in baboons, whereas phenytoin and carbamazepine are only partially active. However, trimethadione, which is active against generalized myoclonic seizures in man, is only weakly active in baboons. Furthermore, single dose administrations of ethosuximide or primidone were found to have little effect on the epileptic response in *P. papio*, although both drugs are active against myoclonic petit mal in humans. Thus, the predictive value of this model for drugs effective against particular types of human epilepsy still requires clarification. Furthermore, the high prime and maintenance

costs of baboons limit the usefulness of this species for drug development.

Photically induced seizures also occur in domestic fowl⁷⁰. However, although the available data suggest that photosensitive fowls are an interesting model for human grand mal epilepsy⁷³, these animals have not yet been used extensively for the evaluation of anticonvulsant drugs. On the other hand, mice with an inborn susceptibility to audiogenic stimulation are widely used in antiepileptic drug evaluation and screening⁷⁷. Most studies on audiogenic seizure-susceptible mice have been performed in the DBA/2 inbred strain of the house mouse (*Mus musculus*), which has been known since 1947 to be susceptible to sound-induced seizures^{12,13,15}. Nearly 100% of the males and females of this strain undergo an age-dependent, often fatal, sequence of violent generalized convulsions when initially exposed to intense auditory stimulation. The seizures begin with a wild running phase, followed by clonic convulsions and a tonic extension, ending in respiratory arrest (in about 60%) or full recovery. The clonic seizures are normally used for evaluation of anticonvulsant drug action. All clinically used antiepileptic drugs protect against clonic seizures in DBA/2 mice⁷⁷. Thus, in contrast to most other known models of epilepsy, sound-induced seizures in DBA/2 mice are not particularly sensitive to a specific clinical category of antiepileptic drugs. Consequently, audiogenic seizure-susceptible mice may be useful as a sensitive gross screening model for potential anticonvulsant drugs, but they cannot predict antiepileptic activity against a specific type of epilepsy. In this respect, it should be noted that sound-induced seizures are exceedingly rare in man and thus the audiogenic seizure susceptible mouse is obviously not a model of a particular human disorder. On the other hand, it is unlikely that a potent anticonvulsant drug would be rejected by this model.

Sound-induced convulsions also occur in rats¹⁶. Seizures in the genetically epilepsy-prone rat (GEPR) are similar to those observed in audiogenic seizure-susceptible mice but, in contrast to mice, rats rarely die after the seizures. For anticonvulsant drug evaluation, the tonic-clonic component of the response pattern in rats is commonly

used⁷⁷. Pharmacological studies have demonstrated that the relative effectiveness of antiepileptic drugs against tonic-clonic seizures in GEPR is very similar to that in the MES test. Thus, the GEPR seems to be a valuable alternative model to the traditional MES test.

Another interesting genetic model is the *Mongolian gerbil*. Reflex epilepsy in this species was first described by Thiessen et al.¹⁷² in 1968, who recognized the trait in animals randomly bred in their laboratory at the University of Texas. In 27% of the gerbils in this colony seizures could be evoked by placing the gerbils in a new environment. Subsequent studies showed that seizures in gerbils can be initiated by various other precipitating environmental stimuli, e.g., onset of bright light, audiogenic stimuli, vigorous shaking of the cage, and different handling techniques³⁹. The most effective stimulus in randomly bred gerbils is air blast (average pressure 5–10 bars, directed at the back of the animals for 15 sec) by which seizures can be evoked in more than 98% of the animals²⁸. The seizure susceptibility to other stimuli, such as handling, can be increased by selective breeding of seizure-susceptible animals¹⁰⁰. With respect to seizure severity, seizures in gerbils can be subdivided into facial myoclonic 'minor' and generalized myoclonic and tonic-clonic 'major' seizures²⁸. Facial myoclonic seizures are predominant in young animals (of about 7–10 weeks), while in older animals seizures are mostly of the generalized myoclonic or tonic-clonic type. Maximum seizure severity is reached at about 7 months and does not change thereafter²⁸. The progressive age-dependent development of seizure severity in these animals represents an interesting parallelity to the age-dependent onset of generalized epileptic syndromes in patients and to the secondary generalization of seizures. The gerbil may therefore be a useful model for study of the mechanism of generalization of epileptic seizures. The pharmacological sensitivity of the various seizure types in gerbils differs in that valproic acid, ethosuximide and benzodiazepines are more potent against 'minor' than against 'major' seizures, while phenytoin, carbamazepine and phenobarbital are inactive or less potent against 'minor' seizures than against 'major' seizures³⁹. Young gerbils with mi-

nor seizures can thus be used as a model for petit mal epilepsy (see Table V), while older animals with major seizures can be used for identifying drugs with efficacy against generalized tonic-clonic seizures. In this respect, it should be noted, however, that it is not yet clear if the seizures in gerbils are primary or secondary generalized, since EEG studies have suggested that the seizures in gerbils might have a focal origin^{29,163}. Unfortunately, there are some drawbacks of the epileptic gerbil model⁷⁷: (1) The sensory stimulation is not specific for epileptic myoclonic seizures but is more often observed in non-epileptic myoclonus¹⁰⁰. (2) The experiments are time-consuming since, due to a long post-ictal refractoriness, the animals can only be tested once a week. More frequent testing results in a marked decrease of seizure severity. Post-ictal refractoriness is also seen in stimulus-sensitive epileptic patients but is also typically seen in non-epileptic myoclonus¹⁰⁰. (3) A certain skill in handling when drugs are to be administered is mandatory in order to avoid the induction of seizures (which would then be followed by post-ictal refractoriness). (4) As in other rodent species the half-lives of antiepileptic drugs (and possibly other compounds) in gerbils are much shorter than in man which renders the maintenance of effective drug levels during chronic treatment difficult²⁸. This problem, however, can be resolved by constant rate infusion of drugs via subcutaneously implanted osmotic minipumps²⁸.

A mouse mutant which may be suited as an animal model for complex partial seizures is the *El* mouse^{41,154}. *El* mice exhibit seizures in response to vestibular stimulation (by repeated tosses into the air or by altering the equilibrium of the mice) which appear to originate in the hippocampus or other deep temporal lobe structures and then spread to other brain regions. The generalized tonic-clonic seizures which occur in these animals are preceded by excessive salivation and head, limb and chewing automatisms. EEG recordings indicate a localized onset of paroxysmal activity¹⁵³. Thus, the seizures in *El* mice can be best classified as complex partial seizures with secondary generalization. Accordingly, *El* seizures can be inhibited completely by phenytoin and phenobarbital, i.e., drugs of choice for treating this type of

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epilepsy in humans¹⁵⁴. Despite these interesting features, the El mouse is not yet widely used in antiepileptic drug evaluation but is primarily employed in the search for seizure mechanisms.

In summary, most of the diverse animal species with genetic predisposition to reflex seizures do either not show advantages to traditional seizure models, such as the MES or PTZ tests, for the search of anticonvulsant drugs, or their predictive value for antiepileptic drug efficacy is not yet clear. However, it should be emphasized that most of the genetic animal models of epilepsy described here may provide information about the fundamental mechanisms involved in the onset and development of the epilepsies that is inaccessible with most other techniques of seizure induction⁷⁷. Furthermore, the canine and baboon models offer unique opportunities for testing the more subtle effects of drugs on behaviour in an experimental paradigm that might permit more accurate prediction of drug effects in man than rodent models. In any event, the application of such models should be reserved for drugs with activity previously demonstrated by testing in standard models in small animal species.

Models with electrical or chemical seizure induction.

In contrast to the various genetic animal models of epilepsy reviewed above, electrical or chemical methods to induce seizures use 'normal,' i.e., not genetically predisposed, animals, usually mice or rats (Table II). With the exception of some models of focal epilepsy (e.g., the aluminium gel model), electrically or chemically induced seizures are models of seizure states rather than models of (chronic) epilepsy. As discussed above, animals which are not seizure susceptible per se might be more responsive to anticonvulsants than are seizure-susceptible animal species, which may lead to an exaggeration of the anticonvulsant potency of a new drug. Nevertheless, with respect to screening purposes, electrically or chemically induced seizures have advantages to most genetic models.

Electrically induced seizures. Three major types of electrical seizure models can be differentiated^{41,104,165}: (1) *threshold models*, in which the current (or voltage) necessary to elicit a minimal

(clonic) or maximal (tonic extension) seizure is quantitated; (2) the *MES test* with supramaximal stimulation; and (3) *focal electrical stimulation*, as done in the *kindling model*, in which repeated stimulation of a brain region with initially subconvulsive electrical stimulation leads to the development of focal and secondarily generalized seizures. With the exception of the minimal electroshock threshold test, which could not be validated as a model for a specific clinical seizure type⁶⁵, these different models will be discussed in more detail in the next sections as valuable tools to identify drugs with efficacy against generalized tonic-clonic and focal seizures. Models which use acute focal electrical stimulation with convulsive stimuli to induce afterdischarges provide a relatively simple method for reproduction of the ictal phenomena of partial epilepsy¹⁰⁴ but, compared to kindling, have the disadvantage that the epileptogenic process cannot be studied.

Chemically induced seizures. Innumerable chemicals induce seizures at toxic doses; only compounds which are of use as tools for epilepsy research will be discussed here (see Table II). Chemoconvulsants induce seizures after both systemic and central (e.g., focal) application. The most commonly used model with systemic administration of a convulsant is the *s. c. PTZ model*. PTZ induces generalized clonic and, in higher doses, tonic seizures after different routes of administration. The seizures are paralleled by spike-wave complexes (clonic seizures) or sharp hypersynchronized poly-spikes (tonic seizures) in the EEG³⁰. By means of i.v. infusion, the threshold doses to the different components of PTZ seizures can be quantitated. Convulsant doses of PTZ after i.p. administration are similar to those after s.c. injection, but latency to the seizures is somewhat shorter. PTZ-induced tonic ('maximal') seizures can be blocked by those antiepileptic drugs which are also effective in the MES test, whereas PTZ-induced clonic seizures are widely used as a model that predicts drugs effective against generalized seizures of the petit mal (absence) type¹⁹⁰. The advantages and disadvantages of the PTZ model of petit mal epilepsy will be discussed in the following sections.

The mechanism of the convulsant action of PTZ seems to be related to the inhibitory function of

TABLE III

Models proposed for evaluation of antiepileptic drugs

For details see text. Abbreviations: PTZ, pentylenetetrazol; CD 97, convulsive dose is 97% of animals tested (as determined from dose-effect curves)

- (1) Threshold for tonic (maximal) electroconvulsions in mice.
- (2) Threshold for clonic seizures after i.v. infusion of PTZ in mice.
- (3) Maximal electroshock seizure test (suprathreshold stimulation with 50 mA in mice and 150 mA in rats).
- (4) s.c. PTZ test (clonic seizures induced by CD 97 of PTZ in mice (about 80–100 mg/kg) and rats (about 70 mg/kg)).
- (5) Amygdala kindling model in rats (separate evaluation of effect on focal (stage 1–3) and generalized (stage 4–5) seizures and amygdalar afterdischarges).
- (6) Determination of "neurotoxicity" (subclinical neurological deficit, such as sedation, impairment of motor coordination, muscle relaxation) by rotarod or chimney test in mice and rats.
- (7) Further (more specialized) models if test drug looks promising.

the neurotransmitter gamma-aminobutyric acid (GABA). PTZ has been shown to have an affinity for the chloride-ionophore of the postsynaptic GABA receptor ionophore complex and to antagonize GABAergic function¹²⁷. There are several other compounds that act as GABA antagonists, such as *bicuculline*, *picrotoxin*, *benzylpenicillin* and the cage convulsants¹²⁷, or impair GABAergic neurotransmission by inhibition of GABA synthesis (for examples see Table III) (cf., refs. 86, 108, 110). Seizures induced by these compounds are in most instances similar to those induced by PTZ so that these chemoconvulsants do not exhibit advantages vs. PTZ for drug screening. In this respect it is interesting to note that parenterally injected penicillin in cats has been proposed as a suitable model of petit mal epilepsy¹¹⁵. However, cats are too expensive for large scale studies, such as drug screening, and penicillin fails to provide a petit mal model in rodents. Irrespective of drug screening purposes, however, convulsants which impair GABAergic neurotransmission by differential mechanisms might be valuable in elucidating the action of an anticonvulsant drug on the functional parts of GABAergic transmission. This also applies to so-called inverse benzodiazepine (BZ) receptor agonists, such as β -CCM, DMCM and FG 7142 (see Table II), which bind to the BZ binding site of the GABA receptor ionophore complex but exert the opposite effects as clinically used benzodiazepines, such as diazepam^{129, 160}. As to be expected, seizures induced by such inverse BZ agonists are blocked by diazepam and related benzo-

diazepines, but interestingly also by valproic acid at doses much lower than those effective in the PTZ test¹²⁹. Besides compounds that induce seizures via an effect on GABAergic neurotransmission, there are several other groups of chemoconvulsants which exert selective effects on specific neurotransmitter systems (Table II), such as glycine antagonists (e.g., *strychnine*), cholinomimetic drugs (e.g., *pilocarpine*) and excitatory amino acid receptor agonists (e.g., *NMDA* or *NMDLA* and *kainate*)^{110, 111, 161, 176}. These compounds are valuable to study seizure mechanisms and the effect of anticonvulsant drugs on these mechanisms. However, their predictive value for specific types of human epilepsy is not clear. Interestingly, both the glycine antagonist strychnine and the glutamate agonist NMDLA (*N*-methyl-D,L-aspartate) induce seizures that are insensitive to standard antiepileptic drugs and it has been suggested that seizures induced by these compounds may be used as models to study mechanisms of drug resistance⁸⁴.

Among the miscellaneous convulsants with other mechanisms of action than those already discussed (see Table II), *gamma-hydroxybutyric acid* (GHB) deserves special comment because seizures induced by this compound in different species have been proposed as a valuable model for petit mal epilepsy^{123, 158}. In rats, administration of GHB or its pro-drug *gamma*-butyrolactone produces hypersynchronous spikes and waves in the EEG which are associated with behavioural arrest and stupor and spontaneous and auditory evoked myoclonic jerks^{123, 159}. As shown in Table V, the

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paroxysmal discharges in the EEG can be blocked by drugs, such as valproic acid, ethosuximide and trimethadione, while phenytoin is inactive³. Interestingly, GHB is a minor metabolite of GABA in the brain and recent evidence suggests that GHB may have neurotransmitter function of its own¹⁵⁹. Valproic acid seems to block the synthesis of this endogenous epileptogen, whereby some convulsants, such as 3-mercaptopropionic acid and kainate apparently enhance its formation¹⁶⁰. Despite the interesting characteristics of the GHB model, it is not yet widely used in antiepileptic drug research. With respect to the other chemoconvulsants shown in Table II, it should be noted that the DDT-induced myoclonus is used as a model of human action myoclonus and not of epileptic seizures¹⁶⁰.

As already mentioned, chemoconvulsants induce (focal) seizures if applied directly onto or into the brain, e.g. by intracerebroventricular injection, application onto or into brain cortex and by injection into specific subcortical brain nuclei¹³⁴. Some examples of drugs which are used in this respect are shown in Table II. Whereas PTZ and *henicillin* seem to induce seizures by antagonism of GABAergic function, seizures after application of kainate and quinolonic acid seem to be a consequence of stimulation of excitatory amino acid (glutaminergic) receptors^{5,18,152}. Similar to the models using systemic administration of chemoconvulsants, these focal models thus simulate selective disturbances of inhibitory and excitatory neurotransmission which might be involved in human epilepsies.

Topical convulsant metals. Focal seizures can also be induced by topical application of certain metals, such as *alumina cream* (aluminium hydroxide), cobalt and tungstic acid, onto (or into) the cortex¹⁵⁷. More recently it was shown that recurrent seizures can also be induced by the injection of iron into the brain cortex¹⁶¹. Topical convulsant metals have proved popular because they provide a model for relating neurochemical changes with seizure states over time¹⁵¹. With cobalt, for example, there typically is a period of several days before seizures are prominent, a period of several days of intense seizure activity, and a period of declining seizure activity. Neurochemi-

cal studies on these models have provided evidence that GABAergic inhibition is impaired at focal sites, which could explain the convulsant effect induced by these metals¹⁶. Of interest in this respect is that impaired GABAergic transmission at focal sites has also been found in patients with temporal lobe epilepsy⁷⁴, which suggests that GABA impairment is a hallmark of seizure foci. However, it should be considered in this respect that topical convulsant metals produce severe damage at sites of application which could account for loss of both excitatory and inhibitory synapses. Similar to human focal epilepsy, the convulsant metal models, especially those using tungstic acid, are relatively resistant to antiepileptic drug therapy. However, none of these models is widely used as a screening model for antiepileptic drug development, although the alumina cream model in monkeys (*Macaca mulatta*) is now employed in the NINCDS program to evaluate candidate drugs which have successfully passed all sequential test phases of the preclinical screening programme¹⁹.

It should be noted that epileptic cortical foci can also be produced by briefly freezing a small area of cerebral cortex¹⁰.

Neurophysiological models. At the end of this brief survey of animal models, more sophisticated neurophysiological seizure models deserve consideration. In these models, single neurones (in intact brain, brain slices or tissue culture) are made 'epileptic' by microiontophoretic application of convulsants, electrical stimulation or by other means of induction of membrane hyperexcitation, such as rearrangement of extracellular ionic environment^{19,11,10,101,106}. These models allow the study of seizure mechanisms and antiepileptic drug actions at the neuronal level and can thus be used to identify new drugs with selective effects on neuronal processes; however, they do not substitute 'whole animal' models for specific seizure types. Furthermore, it should be noted that electrophysiological studies often are carried out in preparations from non-mammalian species, such as snails (*Aplysia californica*, *Helix pomatia*), squids, leeches, crayfish or frogs¹⁰¹. The transferability of data obtained in such species to mammalian central neurones is not easy to judge, especially when peripheral nervous tissues, such as ganglion cells, are

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used in respective electrophysiological preparations. In any event, these preparations provide information on the subtle effects of antiepileptic drugs on excitable tissues that is inaccessible with most other techniques used in characterization of new antiepileptic drugs.

MODELS PROPOSED FOR THE SEARCH AND DEVELOPMENT OF ANTI-PILEPTIC DRUGS

Animal models for antiepileptic drug discovery and development should allow large scale evaluation of drugs in a standardized, consistent manner. Thus, the models which will be proposed in the following are a compromise between the selection criteria for ideal models described at the beginning of the preceding section and the necessity of using models which are generally available have been validated and can be performed with ease. In

order to minimize the possibility that an interesting new anticonvulsant drug is missed, not only models with supramaximal seizure induction, such as the commonly used MES test, or models with administration of fixed doses of a chemoconvulsant, such as the s.c. PTZ test, should be used but also threshold models, in which the effect of the drug on seizure threshold of an individual animal (or group of animals) is determined instead of using a fixed electrical or chemical seizure stimulus which ignores individual differences of animals in terms of seizure susceptibility. By comparison of drug effects in threshold and (supra)maximal models it can be differentiated if anticonvulsant activity results from the elevation of seizure threshold or from other mechanisms, such as reduction of seizure spread¹³³. Furthermore, threshold tests allow detection of proconvulsant effects of a given drug. As emphasized in the Introduction, not only models of generalized seizures should be used, but it is necessary to include at

TABLE IV

Anticonvulsant potency of common antiepileptic drugs

Doses shown to be effective in seizure threshold tests are those which significantly increase the threshold by about 20%. Anticonvulsant ED₅₀'s given are those determined at time of maximal effect. Inactive compounds were tested up to toxic doses. Abbreviations: MES threshold, threshold for maximal (tonic) electroconvulsions; i.v. PTZ threshold, threshold for clonic seizures induced by i.v. pentylene tetrazol; MES test, maximal electroshock seizure test (supramaximal stimulation); s.c. PTZ test, evaluation of effect on clonic seizures after subcutaneous administration of PTZ; ADD 1, decrease in duration of amygdalar afterdischarges; TD₅₀, median neurotoxic dose as determined in the rotarod or chimney test; SRF ↓, reduction of sustained repetitive firing; GABA ↑, increase in GABAergic transmission; FS, focal seizures; GTCS, generalized tonic-clonic seizures; GAs, generalized absence seizures; IS, infantile spasms; NE, not effective. Data are from refs. 33, 39, 50, 54, 67, 79, 94, 102, 112, 141 and unpublished experiments of our group.

Antiepileptic drug	Presumed mechanism of action	Doses effective to increase seizure threshold in mice (mg/kg i.p.)	Anticonvulsant ED ₅₀ (mg/kg i.p.)						Neurotoxicity (TD ₅₀ bi mg/kg i.p.)		Antiepileptic effect in humans			
			MES test (gen. tonic seizures)		s.c. PTZ test (gen. clonic seizures)		Amygdala kindling in rats		Focal seizures		Gen. ADD		Mice	Rats
			Mice	Rats	Mice	Rats	Gen.	Gen.	ADD	ADD				
Phenacetoин	SRF 1	5 ^a	NE	10	14	NE	NE	50	30	1 ^c	65	130	FS, GTCS	5-6
Carbamazepine	SRF 1		NE	9	4	NE	NE	15	8	1	72	26	FS, GTCS	13-20
Phenobarbital	GABA ↑?	1.5 ^a 4 ^a	22	12	13	7	44	16	1	69	24	FS, GTCS	2-3	
Primidone	?	2.5 ^a	11	17	60	30 ^a	>100 ^a	>100 ^a	NE ^a	680	>300	FS, GTCS	10-15	
UAlprolid acid	SRF 1	110	100	270	100	150	74	320	190	↓	425	365	GAS, GTCS.	20-50
	GABA ↑?												IS (?) , FS (?)	
Ethoxzolamide	?	NE	160 ^a	NE	NE	130	54 ^a	NE ^a	310	NE ^a	440	220	GAS	13-20
Diazepam	GABA ↑	3.5 ^a 0.5 ^a	19	5	0.2	0.3	>10	1.4	1	7.3	2.4	2.4	Status.	-0.31.v
Clonazepam	GABA ↑		22 ^a	1.2	0.009	0.06 ^a	>1	0.06	↓	0.2	0.4	0.4	FS, GTCS, GAS (tolerance)	0.1-0.2
													status	-0.031.v

^b active after chronic administration; ^c in some animals increase in ADD; ^d up to 400 mg/kg.

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TABLE V

Comparison of anticonvulsant potency of common antiepileptic drugs and some GABA mimetic drugs in different animal models of petit mal seizures

In rats with spontaneous petit mal seizures, ED₅₀'s are those doses which decrease by 50% the duration of spike/wave discharges in the 60 min period after drug administration. In tottering mice, effective doses are those which decrease the number of spike-wave discharges. In the GHB model in rats, effective doses are those which completely antagonized paroxysmal EEG activity (except for phenobarbital, which at the dose indicated counteracted seizure activity in the EEG in only 30% of the animals). Spike-wave discharges in genetically predisposed rats and tottering mice are accompanied by a sudden arrest in movement, twitching of the vibrissae and single myoclonic jerks of face, head or neck. In the GHB model, paroxysmal EEG activity is accompanied by behavioural stupor and myoclonic jerks. Abbreviations: PTZ, pentylenetetrazol; GHB, gamma-hydroxybutyric acid; NE, not effective; THIP, 4,5,6,7-tetrahydro-dioxazolo(5,4-c)pyridine-3-ol. Data are from refs. 35, 43, 48, 79, 96, 103, 116, 117, 122, 141, 158 and Table IV.

Drug	Effective doses (mg/kg i.p.) in experimental models of petit mal seizures							GHB model in rats (spike and wave discharges)	Efficacy against absence seizures in humans	Efficacy against myoclonic seizures in humans	Clinical dose (mg/kg day p.o.)				
	Genetic animal models				s.c. PTZ model (clonic seizures)										
	Rats with spike-wave discharges (ED ₅₀)	Tottering mice with spike-wave discharges	Gerbils with facial myoclonic seizures (ED ₅₀)	Baboons with photo-myoclonic seizures	Rats (ED ₅₀)	Mice (ED ₅₀)									
Valproic acid	70		210 ^a	200 ^b	74	150	300	+++	+++	20-50					
Ethoxzolamide	15	150	360 ^a	NE	34 ^a	130	300	+++	+++	15-20					
Trimethadione	70			weakly active	110 ^a	300	300	+++	++	20-40					
Diazepam	0.7	1.4	0.25	0.5-1 ^a	0.3	0.2		+	+		not used				
Cliazepam			0.029	0.15 ^a	0.06 ^a	0.009		+	+		0.1-0.2				
Phenobarbital	NE ^c	NE	NE	15-50 ^a	NE	NE	NE	NE	NE						
Carbamazepine	NE ^c		~40 ^a	40 ^a	NE	NE	NE	NE	NE						
Phenobarbital	2 ^c	25	14 ^a	15 ^a	7	13	30 ^a	NE	++	2-3					
Primidone				NE	20 ^a	60	NE	NE	++	10-15					
Progabide	NE ^c		58	60 ^a	NE		?		?						
γ -Vinyl GABA (vigabatrin)	NE ^c			450-950 ^b	7 ^a	940	NE ^c	?	?						
THIP (gaboxadol)	NE ^c		1	NE ^c	NE		?	?	?						

^ap.o.; ^bi.v.; ^cdevelopment of tolerance; ^ain higher doses aggravation of seizures; ^bonly partially effective; ^canticonvulsant effect lost at higher doses (20 mg/kg); ^dpreliminary data indicate that the compound is not effective; ^evigabatrin aggravates i.v. induced PTZ seizures in rats¹²³.

least one model of focal seizures in antiepileptic drug development. Based on pharmacological validation of the diverse focal seizure models, the kindling model was chosen for this purpose. We also need models for drug-resistant seizure types, such as myoclonic astatic seizures or infantile spasms, but no specific models for these seizure types are available yet. Two species, i.e., mice and rats, are used in the models proposed in the following since, as will be illustrated, potentially useful compounds may be missed if only one species is used in preclinical evaluation. Furthermore, as shown in Tables IV and V, active doses of antiepileptic drugs in rats do more closely reflect the clinical potency of the respective drugs than those in mice. Not only models for anticonvulsant activity

should be used in the early phases of drug development but also models for detection of central side-effects, in order (1) to determine if the anticonvulsant action of a test compound is a selective effect or is only secondary due to other effects, such as impairment of motor function, and (2) to determine the spread between doses with anticonvulsant action and those which induce undesired side-effects. The models which we propose for these different aspects of antiepileptic drug evaluation are shown in Table II.

Threshold for maximal (tonic extension) electro-convulsions in mice

This is a sensitive test to determine the ability of a drug to alter the seizure threshold for tonic limb

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extension¹⁶⁵. Antiepileptic drugs with clinical efficacy against generalized tonic-clonic seizures all increase this threshold, while drugs, such as ethosuximide and trimethadione, which are clinically ineffective against this seizure type, do not increase the threshold in non-toxic doses (see Table IV). This test thus predicts drugs effective in generalized seizures of the grand mal type and is much more sensitive in this respect than the traditional MES test with supramaximal seizure induction. Electric stimulation is applied via corneal or ear electrodes with a stimulator that either delivers constant current or constant voltage at a frequency of 50–60/sec. A stimulus duration of 0.2 sec is commonly used. Both sinusoidal or rectangular pulses can be used for stimulation, although the latter are more efficient in producing seizures. An important point is the internal or serial resistance of the stimulator which should be chosen in consideration of the external resistance of the animal (about 5 kΩ). We usually switch the serial resistance of the stimulator to 10 kΩ for both mice and rats. With constant current stimulators, it is advantageous to use a powerful apparatus with self-adjusting stimulus voltage (according to the impedance of test object), thus ensuring the application of the present current. The threshold is usually determined as the current or voltage inducing hind limb extension in 50% of the animals. In rat strains in which hind limb extension cannot be induced reliably by electrical stimulation, forelimb extension can be used as an end-point. The CC₅₀ (convulsant current in 50% of the animals) or CV₅₀ (convulsant voltage in 50% of the animals, also designated as EV₅₀) can be determined from current (or voltage)-effect curves corresponding to dose-effect curve determinations⁷² by using 8–10 animals per stimulus intensity. Much less time and animals are needed for threshold determination by so-called 'up-and-down' methods^{42, 62} by which the EV₅₀ and confidence limits for 95% probability can be determined in a single group of 15–20 animals. The principle of these methods is that the stimulus intensity for each animal is determined by the response of the animal just tested. Thus, stimulus intensity is increased to the next pre-fixed higher increment if the previous animal failed to exhibit a tonic seizure and to the next lower increment if the

animal exhibited a seizure. The EV₅₀ can then be calculated mathematically by the positive (tonic seizure) and negative (no tonic seizure) responses obtained within the group of animals⁶². Statistical tests, such as Student's *t* test, can be used to calculate the significance of drug effects. For comparison of drug effects, it was suggested to calculate the dose which elevates the threshold by 20%¹⁶⁸, which can be done by plotting the doses of the respective drug against the percentage threshold increase on a semilogarithmical scale⁷³. Respective doses of antiepileptic drugs are shown in Table IV. Since the control threshold in mice varies as a result of age and daily (circadian) or hormonally induced rhythms, control threshold determinations should be undertaken on each day parallel to threshold determinations in drug-treated animals. Control thresholds in mice are about 6–9 mA or 90–140 V, respectively, depending on strain, age and method of stimulation (thresholds determined via ear electrodes are somewhat lower than those determined via corneal electrodes). If animals are used more than once, the post-ictal rise in seizure threshold should be considered. If determined not more than once daily, the seizure threshold is remarkably constant.

Threshold for clonic seizures after i.v. infusion of PTZ in mice

In this test, timed intravenous infusion of PTZ is used to calculate the threshold dose of PTZ which induces a clonic seizure. When PTZ is administered by continuous i.v. infusion, overt seizure activity appears as follows: the animal first exhibits one or more isolated jerks ('first twitch') immediately followed by a generalized clonic seizure with loss of righting reflexes and, after a certain time lag, by a maximal tonic-clonic seizure. All 3 seizure components have been used as end-points in the evaluation of anticonvulsant drugs by this test¹⁶⁶, but it should be noted that the time to the onset of tonic activity is often variable and thus less suited for drug evaluation. Threshold doses of PTZ for the different seizure components depend on the rate of infusion and the concentration of PTZ in the solution^{57, 73}. With infusion of a 1% solution at 0.3 ml/min, the threshold dose for the generalized clonic seizure with loss of righting re-

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flexes (the most reliable end-point) is about 50 mg/kg, that for the maximal tonic-clonic seizure about 90 mg/kg. Since even brief physical restraint lowers seizure threshold, methods to allow infusion into the tail vein of unrestricted freely moving mice should be used⁴⁹. Eight to 10 animals are used per threshold determination. The threshold is calculated as mean dose (\pm S.D.) of PTZ that induced the seizures in the group tested. The i.v. PTZ threshold test for clonic seizures is a sensitive measure that seems to predict drugs effective in generalized seizures of the petit mal type, which is illustrated by the efficacy of ethosuximide and valproic acid and the inefficacy of phenytoin and carbamazepine in this test (Table IV). In this respect, the i.v. PTZ threshold test is a more sensitive predictor than is the s.c. PTZ test, as will be seen later in this review.

Maximal electroshock seizure (MES) test in mice and rats.

The same stimulation device that is used for determination of the MES threshold can be used for this test, but the strength of the stimulus should be increased to at least 2 (but better 4–5) times the threshold current. All animals are stimulated with the same supramaximal current strength. With constant current (50–60/sec) stimulators, 50 mA should be used for mice and 150 mA for rats^{164, 166, 168}. With constant voltage stimulators, at least 250 V should be used for mice and 750 V for rats (threshold for maximal seizures in rats is about 250–350 V or 20–50 mA, depending on strain and age). Especially in rats, control determinations are necessary because some strains (and old animals) are quite resistant to electroshock stimulation. When necessary, forelimb extension is an alternative end-point. The stimulus is applied via corneal or ear electrodes for 0.2 sec. For resistance of the apparatus see above. If a lower stimulus strength than that indicated above is used, the test is not longer supramaximal and data obtained in this way are not longer comparable (efficacy of anticonvulsant drugs increases with decreasing stimulus strength). Anticonvulsant potency of a drug in the MES test is determined by calculation of its anticonvulsant ED₅₀ for suppression of tonic hind limb extension. Groups of 8–10 animals are

used per dose and the ED₅₀ is calculated from the dose–effect curve, e.g., by the method described by Litchfield and Wilcoxon⁷². The MES test is probably the best validated test that predicts drugs effective in generalized tonic-clonic (grand mal) seizures. As shown in Table IV, drugs, such as phenytoin, carbamazepine, phenobarbital and primidone are highly active in this test; while ethosuximide is ineffective. However, due to the strong seizure stimulus, the test may yield false negatives (see discussion of GABA mimetic drugs below) in that potential clinically effective anticonvulsants are rejected at an early state of development. This can be avoided by parallel use of the MES threshold test described above.

s.c. PTZ test in mice and rats

In this test, the s.c. CD 97 (convulsive dose in 97% of the animals) of PTZ for induction of clonic seizures is administered (approximately 10 min before the anticipated time of peak anticonvulsant drug activity) and animals are observed for 30 min after PTZ injection for the occurrence of clonic seizures^{164, 166, 168}. As end-point, either the first episode of clonic jerking which persists for at least a 5 sec period (a 'threshold seizure') or (possibly more reliable in gross screening) the first clonic seizure with loss of righting reflexes is taken. Transient myoclonic jerks are not considered as constituting a convulsion. Doses to induce clonic seizures may vary from strain to strain, but are in most instances about 70 mg/kg in rats and 80–100 mg/kg in mice. Control determinations (or better determination of CD 97) should be done, especially in rats, prior to drug efficacy experiments (at least once). Anticonvulsant ED₅₀s for suppression of clonic seizures (either threshold seizure or clonic seizure with loss of righting reflexes) are determined by using 8–10 animals per dose. ED₅₀ is then calculated from the respective dose–effect curve⁷². The s.c. PTZ test is widely used as a standard model for petit mal epilepsy. As shown in Table IV, ethosuximide, valproic acid and the benzodiazepines are active in this test, while phenytoin and carbamazepine are not. However, it should be noted that phenobarbital and primidone block PTZ seizures much more potently than do ethosuximide and valproic acid, which might suggest that the PTZ test is

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model for myoclonic rather than absence seizures (see detailed discussion in the last section of this review). There are several other (chronic) models of petit mal epilepsy that may be better suited as petit mal models than the simple PTZ test (see Table V). However, although the s.c. PTZ test is certainly not the best available model of petit mal, for the sake of simplicity it is still proposed as a screening model. Limitations with respect to results obtained with GABA mimetic drugs in this test will be discussed later in this review.

Amygdala-kindling model in rats

The kindling phenomenon is a manifestation of the fact that 'epilepsy induces epilepsy'⁶⁴. Evidently through a kind of positive feedback mechanisms, a local epileptiform discharge, confined initially to a small focus or network, tends, if not disturbed (e.g., by treatment with antiepileptic drugs), to spread in space and severity. Goddard et al.⁴³ characterized this phenomenon in detail and in a systematic manner, which they came to refer to as 'kindling.' Animals chronically implanted with stimulation and recording electrodes in one structure of the limbic system or other brain areas (the amygdala being the most responsive structure) develop seizures upon periodic electrical stimulation with an initially subconvulsive current. For stimulation, either a fixed current strength is used (e.g., 400–500 μ A 1 msec monophasic square-wave pulses for 1 sec with 50 or 60/sec) or the individual threshold current to induce afterdischarges at the site of stimulation. In any event, the electrical stimulus must induce local afterdischarge in order for kindling to develop. During repeated (usually once daily) stimulation of the amygdala with this current seizures develop, which generally evolve through the following 5 classes¹²⁸: (1) immobility, eye closure, twitching of vibrissae, stereotypic sniffing, (2) facial clonus (e.g., chewing) and head nodding, (3) unilateral forelimb clonus (contralateral to focus), (4) rearing, often accompanied by bilateral forelimb clonus, and (5) rearing with loss of balance, and falling accompanied by generalized clonic seizures. All of these classes are associated with reduced responsiveness to sensory stimulation in comparison to the normal waking state. The behaviour ob-

served in classes 1 and 2 mimics that found in human complex partial (limbic or temporal lobe) seizures which, as amygdala-kindled seizures, most often originate from foci within the limbic system (including the amygdala); class 3 is still a focal seizure, which mimics a simple partial seizure in human epilepsy, while the latter 2 classes represent secondary generalized motor seizures¹⁰⁵. Once the enhanced sensitivity (as evidenced by class 5 seizures) has developed, the animal is said to be fully kindled. The increased sensitivity persists for at least months after kindling has been established and thus appears to reflect persistent changes in brain function.

Most kindling experiments are terminated after elicitation of a single or several class 5 motor seizures. Pinel and Rovner¹³² found, however, that continued periodic stimulation of the amygdala or other brain regions of the rat leads to spontaneous motor seizures. Among those manifesting at least 3 spontaneous class 5 seizures, the occurrence of spontaneous seizures persisted for as long as 7 months following termination of the stimulation, suggesting that epilepsy with spontaneously recurring seizures was induced. Spontaneous seizures induced by kindling have also been identified in other species, including baboons, cats, and dogs¹⁰⁵.

Engel and Cahan²⁴ recently reviewed the potential relevance of kindling to human partial epilepsy. They pointed out that there is no evidence from clinical data to evoke kindling mechanisms as a means of creating an epileptogenic lesion. However, progressive changes in human brain can be demonstrated that could possibly be explained by kindling in the human brain. For instance, trans-synaptic electrophysiological changes are similar in amygdala-kindled animals and in human limbic epilepsy and initial behavioural manifestations suggest the same structures (amygdala and hippocampus) are involved.

Procedures and mechanisms of kindling have been reviewed in 3 volumes^{184–186} and various reviews^{6, 42, 105, 107, 131}. Kindling is a relatively time-consuming procedure as it requires the chronic implantation of stimulation and recording electrodes and regular electrical stimulation (with once daily amygdala stimulation approximately 10–15 days)

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till establishment of fully kindled seizures, which usually are taken for antiepileptic drug evaluation. If animals are kindled too rapidly (e.g., by several stimulations per day), the post-ictal refractory state produced by each stimulation interferes with kindling acquisition². Furthermore, if the recovery period after electrode implantation is too short (less than 1-2 weeks), the sensitivity of the animals to kindling is lowered.

The kindling model has the merit that the efficacy of the drug against the progressive process leading to epileptogenesis as well as against the fully kindled state can be measured. Furthermore, in fully kindled animals effects of drugs on seizure threshold (about 50-100 μ A in amygdala-kindled rats if kindling was accomplished by once daily stimulation) and seizures induced by suprathreshold stimulation (e.g., by 500 μ A) can be compared as a means to differentiate between drugs which increase seizure threshold and those which act by limitation of seizure spread. Besides effects on seizures induced by threshold or suprathreshold stimulation, 4 different measures for drug efficacy can be recorded in a kindled animal: (1) seizure latency (time from stimulation until the first sign of seizure activity), i.e., a measure of the rate of seizure spread from the focus, (2) seizure severity (graded according to the 5 classes described above), (3) seizure duration, and (4) afterdischarge duration, i.e., a measure of limbic electrographic activity recorded from the focus. An added advantage is that by use of this model chronic drug efficacy studies can be performed with small groups of animals, because in fully kindled rats daily stimulation is possible without altering the seizure parameters³.

Using suprathreshold stimulation in fully amygdala-kindled rats, 2 different approaches for evaluation of drug effects on kindled seizure activity are possible⁴: the usual approach is to measure the effect of the drug on mean latency, severity, and duration of the behavioural seizures as well as mean duration of amygdalar afterdischarges in a group of 8-10 animals. Readings after single dose drug administration are compared with control readings taken 2-3 days prior to and/or after drug injection. Data calculated by this approach do not allow to differentiate between drug activity on focal and generalized components of the behaviour-

al seizures. The second approach is to determine separate ED_{50} values for total suppression of (1) generalized seizures (class 4 and 5), (2) focal seizures (class 1-3), and (3) amygdalar afterdischarges. When animals are tested by the second approach, the generalized seizures provide a valid model for the secondary generalized seizures of partial epilepsy, while the focal components of kindled seizures provide a suitable model for complex partial seizures⁵. Anticonvulsant ED_{50} values obtained against the different seizure types can be easily compared with those obtained in other models of epilepsy. A respective comparison is possible in Table IV and Fig. 2. The drugs of choice against complex partial seizures in man, i.e., phenytoin, carbamazepine, primidone and phenobarbital, are also effective in blocking focal seizures in the kindling model, although it should be noted that primidone, due to accumulation of its metabolite phenobarbital, is only active after prolonged administration (Löscher, in prep.). More recent experiments with phenytoin showed that while the drug is effective in blocking focal amygdala-kindled activity in rats, some animals are totally resistant to phenytoin's action, although plasma levels of the drug are the same as in animals in which the drug is effective (Löscher, in prep.). Such animals represent ideal models for the study of mechanisms of drug resistance. As shown in Table IV and Fig. 2, valproic acid is also effective in blocking kindled focal seizures. Clinically, valproic acid has been shown to be as effective as carbamazepine⁶ and phenytoin^{7,8} for partial seizures in previously untreated patients with epilepsy, while valproic acid seems to be less effective for chronic epilepsy with complex partial seizures. Benzodiazepines are not able to block focal kindled seizures completely, but they reduce seizure and afterdischarge duration⁹. In contrast, ethosuximide is totally ineffective against kindled focal amygdaloid seizures³ (unpublished data of Löscher). In terms of ED_{50} values, carbamazepine, phenytoin, and phenobarbital were much less potent in blocking focal seizures than in blocking generalized motor seizures (Table IV and Fig. 2). Similar data have also been reported by Albright and Burnham². These data are thus consistent with the clinical findings in that complex par-

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tial seizures are more resistant to antiepileptic drug treatment than are generalized seizures¹⁴. As shown in Fig. 2, the secondary generalized kindled seizures were also less sensitive to these drugs than primary generalized (MES) seizures. These data suggest that the kindling preparation is useful in finding new antiepileptic drugs with better efficacy against temporal lobe epilepsy with or without secondary generalization.

Determination of 'neurotoxicity' in mice and rats

Acute toxicity from antiepileptic drugs in rodents almost invariably is manifested by neurological deficits. These include sedation, altered motor activity, ataxia and impaired righting reflexes. These effects of antiepileptic drugs are often summarized by the term 'neurotoxicity,' which, however, is somewhat misleading, since

the sedative/hypnotic effects of some antiepileptic drugs, i.e., phenobarbital and benzodiazepines, are clinically utilized. Minimal neurological deficit, such as impaired motor function, can be detected and quantitated by standardized tests, i.e., by the rotarod test²⁶, in which the animals are placed on a rotating plastic rod (which rotates at a speed of 6 rev/min for mice or approximately 8 rev/min for rats; rods of different diameter have to be used for mice and rats), or by the chimney test⁸, in which the animals have to climb up backwards in a glass or plastic tube (25 cm length and 3 cm ID for mice of about 25–30 g or 50 cm length and 6 cm ID for rats of about 200–300 g). Neurological deficit in the rotarod test is indicated by inability of the animal to maintain its equilibrium for 1 min on the rotating rod or, in the chimney test, by the inability of the animal to climb up backwards in the tube

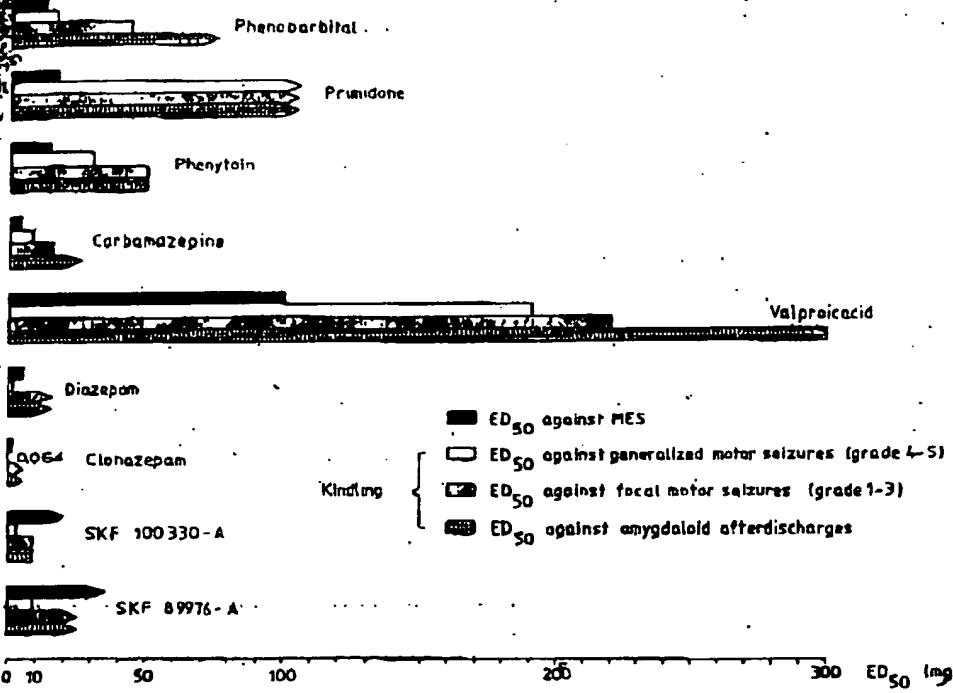


Fig. 2. Comparison of anticonvulsant potencies of various antiepileptic drugs in the MES and amygdala-kindling models. Bars indicate ED_{50} for total suppression of the respective seizure type in rats. Pointed bars indicate that the drug was inactive or only partially active up to the dose shown by the bar. SKF 100330-A and SKF 89976-A are inhibitors of GABA uptake (see Table VI and text). Data are from Löscher et al.¹⁴.

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within 30 sec. The median minimal 'neurotoxic' dose (TD_{50}), i.e., the dose which induces minimal neurological deficit in 50% of the animals, is determined by construction of dose-effect curves using 8-10 animals per dose⁷². Both tests give similar TD_{50} values for a respective compound. An alternative technique for determination of impaired motor function, i.e., the 'inverted screen test' (which evaluates the number of mice which either fall off an inverted screen or do not climb to the top of the screen within 60 sec after inversion), has been described by Coughenor et al.¹⁷. 'Protective indices' for anticonvulsant drugs are obtained by calculating the ratio between TD_{50} and anticonvulsant ED_{50} , which reflects the spread between the median effective dose and the median minimal neurotoxic dose⁶⁶. As can be calculated from the data in Table IV, protective indices for clinically used antiepileptic drugs determined after parenteral (i.p.) administration vary between about 2 and 60, depending on drug, species and seizure test used. New anticonvulsant drugs which in mice and rats exert anticonvulsant effects only at 'neurotoxic' doses ($ED_{50} \geq TD_{50}$) are considered as non-selective and should not be proceeded to further evaluation. For a promising test drug, additional tests to determine the acute toxicity profile have been described elsewhere¹⁶⁷.

Further (more specialized) models if test drugs look promising

Drugs which have shown anticonvulsant activity at non-toxic doses in the tests described above should be further characterized in more sophisticated models (see Table II), such as epileptic strains of animals and chemical or neurophysiological models, to delineate more precisely the antiepileptic potential of the candidate substance and to obtain some insight as to its possible mechanism of action. The choice of models will depend on the anticonvulsant profile of the candidate drug at this stage. Information obtained in the models proposed above and eventually also in additional models must be carefully reviewed before one can determine if the accumulated experimental data are sufficiently promising to warrant moving the candidate substance into costly pharmacokinetic and chronic toxicity and teratogenicity studies.

How drugs which look promising in preclinical evaluation systems can eventually be evaluated clinically has been proposed elsewhere¹⁴⁷. Some additional points which are important in the pre-clinical evaluation of antiepileptic potential of a test drug are delineated in the following.

Parenteral vs. oral administration

Clinically established antiepileptics are usually given by the oral route of administration. Consequently, newly developed drugs should be active after oral administration. However, preclinical testing should be started with parenteral, usually intraperitoneal, administration and only in case of activity by this route the efficacy of the compound after oral administration should be studied. Bioavailability after oral administration is then reflected by the ratio between oral ED_{50} and i.p. ED_{50} . It should be noted, however, that in case of marked first pass metabolism, bioavailability will be low after both oral and i.p. administration. Many compounds are not adequately absorbed from the gastrointestinal tract when orally administered in small animal species in aqueous solution or other simple vehicles, but in several instances absorption can be enhanced pharmaceutically by altering the formulation in which the drug is given. Development of respective preparations may be time-consuming and is only warranted if the drug is active after parenteral injection. Some of the antiepileptic drugs shown in Table IV are also only absorbed to a limited extent from the gastrointestinal tract of rodents when given in simple aqueous solution; examples are phenytoin in rats⁵⁵ and valproic acid in mice⁶⁷. Since with such drugs the oral bioavailability may be negatively correlated with the dose administered, false conclusions with respect to protective indices after oral administration may be drawn. For instance, for phenytoin the ratio between the i.p. TD_{50} and ED_{50} by the MES test in rats is 9.2 (Table IV), whereas after oral administration this ratio is higher than 100, because phenytoin is adequately absorbed after the relatively low anticonvulsant doses but bioavailability markedly decreases at the high doses administered for neurotoxicity testing¹⁶⁷. False conclusions from such data can be circumvented by plasma level determinations after i.p. and oral administration.

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Time of peak drug effect

This should be established as early as possible in preclinical testing to avoid false conclusions in the different animal models. For example, phenytoin was initially thought to be ineffective in the kindling model^{1,10}. However, in these studies phe-

nytoin was tested 30 min after administration. More recent studies, in which the efficacy of the drug in the kindling model was examined at time of peak drug effect in rats (1 h) disclosed significant anticonvulsant potency, as could be expected from a model of focal epilepsy⁹⁴. Another example is

TABLE VI

Anticonvulsant potency of new anticonvulsants drugs which presumably act via potentiation of GABAergic transmission

For explanation see Table IV. Abbreviations not explained in Table IV: GABA-T, GABA aminotransferase (the GABA degrading enzyme); BZ, benzodiazepine receptor; n.t., not tested; THIP, 4,5,6,7-tetrahydroisoxazolo[5,4-c]pyridine-3-ol; SKF 100330-A, N-(4-diphenyl-3-butenyl)-guvacine; SKF 89976-A, N-(4-diphenyl-3-butenyl)-nicoic acid; ZK 93423, 6-benzyl oxy-4-methoxymethyl- β -carboline-3-carboxylate ethyl ester; ZK 91296, 5-benzyl oxy-4-methoxymethyl- β -carboline-3-carboxylate ethyl ester; ZK 95962, 5-isopropoxy-4-methoxymethyl- β -carboline-3-carboxylate ethyl ester. Data are from refs. 25, 75, 79, 90, 94, 113, 114, 119, 130, 155, 192 and unpublished experiments of our group.

Anticonvulsant drug	Presumed mechanism of action	Doses effective to increase seizure threshold in mice (mg/kg i.p.)	Anticonvulsant ED ₅₀ s (mg/kg i.p.)						Neurotoxicity (TD ₅₀ in mg/kg i.p.)	Antiepileptic effect in humans			
			MES (gen. tonic seizures)	Mice	Rats	s.c. PTZ (gen. tonic seizures)	Mice	Rats	Amygdala kindling in rats	Focal	Gen.	ADD	Mice
		MES	i.v. PTZ										
γ Vinyl GABA (vigabatrin)	Inhibition of GABA-T	1000 ^a	490	>2000	940	>1200	>1200	1	1370	FS, GTCS	50		
Progabide	GABA agonist	70	78	140	>300	>200	>200	1	240	FS (equivalent)	20-60		
THIP (gaboxadol)	GABA agonist	20	2	>20	>10	>20	>20	NE	4.7	inactive (1 study)	1-2		
SKF 100330-A	GABA uptake blocker			>200 ^b	>15 (11%)	>25 ^b 2	9	3 1	Less sedative than diazepam	NE			
SKF 89976-A	GABA uptake blocker			>100 ^b	>30 (60%)	>40 ^b 6	>20	9 1	Less sedative than diazepam	NE			
Clobazam	Full BZ agonist			33	25	1.2 0.8			135 12	FS (but tolerance)	0.5		
ZK 93423 (6-BOCE)	Full BZ agonist	-0.1	20	0.6	>5	<5 1	△ Diazepam		NE	NE	NE		
ZK 91296 (BOCE)	Partial BZ agonist	-3	NE	15	NE	NE	NE	NE	>150	NE			
ZK 95962 (POCE)	Partial BZ agonist		NE	20	NE	1	NE	<Diazepam	NE				

^ato; ^b much more active after prolonged administration (due to accumulation of GABA by irreversible GABA-T inhibition);
 Production of photosensitivity.

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valproic acid, which is usually administered 0.5–1 h prior to anticonvulsant testing, but time of peak drug effect after i.p. injection in mice and rats is between 5 and 15 min⁷⁸. In order to avoid underestimation of drug efficacy, all analyses should therefore be conducted at the time the candidate drug exhibits peak activity. Peak activity may be determined by any of the models proposed above (also those for minimal neurotoxicity). For instance, if an ED₅₀ or TD₅₀ has already been determined (this is often done 30–60 min after i.p. injection), this dose is then given to several groups of 8–10 mice or rats and 1 group is subjected to the respective test at each 0.25, 0.5, 1, 2, 4, 8, 16 and 24 h after drug administration until the time of peak effect has obviously been passed. With some newly developed drugs, e.g., vigabatrin, peak drug effect in some models is even not reached within 24 h after single-dose administration⁴⁷! An added advantage of determination of time of peak drug effect is that information on the duration of anticonvulsant action of a test drug is obtained.

Acute vs. subchronic efficacy testing

With promising candidate drugs, also subchronic efficacy studies with at least 2 weeks of daily administration should be undertaken. The reason for this is at least 4-fold: (1) With some drugs the anticonvulsant efficacy increases during prolonged treatment; examples are primidone (due to accumulation of phenobarbital), valproic acid (reasons are unknown) and the new antiepileptic drug vigabatrin (due to accumulation of GABA by irreversible inhibition of its degradation). Consequently, determination of acute potency of such drugs underestimates their potency during prolonged treatment and, in case of test compounds, may thus lead to false decisions with respect to further development. (2) With some drugs, especially benzodiazepines, the anticonvulsant efficacy decreases during prolonged treatment due to development of adaptive processes ('functional tolerance') in the brain. Tolerance is clinically advantageous when it concerns the sedative or muscle relaxant side-effects of anticonvulsant drugs but disadvantageous when it involves the anticonvulsant effect itself. In mice and rats, tolerance to the anticonvulsant effect of benzodiazepines can be dem-

onstrated with any of the models proposed above for acute studies within 1–2 weeks of administration^{34,91}. (3) Subchronic administration may give indices for the possible development of drug dependence when the animals are closely observed (body temperature, body weight, seizure threshold, etc.) for some days after drug withdrawal. (4) By use of models, such as kindling, in which the progressive process leading to epileptogenesis can be studied, subchronic treatment may identify drugs that provide prophylaxis against the development of epilepsy following head injury.

For subchronic efficacy testing of anticonvulsant drugs, it is especially important to consider the pharmacokinetics of the respective drug in the species used. Many drugs are eliminated much more rapidly in rodents than in man so that for maintenance of active drug concentrations either the drug has to be administered several times daily or special administration techniques have to be used, such as constant rate application via subcutaneously implanted osmotic minipumps or subcutaneous injection of special depot preparations. Administration via the drinking water is not advantageous for drugs with short elimination half-lives, because rodents drink mostly during the night so that drug levels during day time may be too low for chronic efficacy testing. For drugs which are eliminated not too rapidly, however, administration via the drinking water (or food) may be a very convenient way of prolonged drug application.

ANTICONVULSANT EFFICACY OF NEWLY DEVELOPED ANTICONVULSANT DRUGS IN THE MODELS PROPOSED FOR PRECLINICAL DRUG EVALUATION

Partly as a result of the NINCDS program of the NIH (U.S.A.), numerous anticonvulsant drugs have been developed during the last 10 years, and a certain number of these compounds have recently progressed from preclinical testing to the initial stages of clinical evaluation¹¹³. Three approaches to the development of new anticonvulsant drugs have been used: (1) The old method of finding anticonvulsants involves the screening of all newly synthesized compounds to identify antiseizure ac-

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tivity in rodent models. It was by screening of phenyl derivatives for anticonvulsant properties that phenytoin was discovered¹³⁷ in 1937. (2) A second approach is to modify the chemical structures of existing drugs in order to increase their therapeutic efficacy. In this way also compounds with clinical applications totally different from that of the parent compound may be identified. For instance, it was through exploration of molecular structures related to phenytoin that the anticonvulsant actions of the oxazolidinediones (e.g., trimethadione) and the succinimides (e.g., ethosuximide) were discovered. (3) A more recent approach is based on increased understanding of neurotransmission and the pathophysiological changes underlying seizures. Most attention so far has been focused on the hypothesis that the underlying neurochemical defect in epilepsy may be a functional impairment in the inhibitory GABAergic mechanisms^{74,85,110}. This hypothesis has led to the development and testing of several compounds, i.e., so-called GABAimimetic drugs, which increase GABAergic function. Some of these compounds, most notably vigabatrin and progabide (halogabide), will be reviewed in the following. In this respect, it should be noted that there is some evidence indicating an involvement of GABA also in the mechanisms of action of several clinically established antiepileptic drugs, such as benzodiazepines, valproic acid and barbiturates, while others, such as phenytoin and carbamazepine, seem to act independent of GABAergic processes primarily by suppression of neuronal hyperexcitability via direct effects on neuronal membrane ion conductances¹⁰². In this respect it should be noted, however, that the information on presumed mechanism of action in Table IV and all subsequent tables gives only a direction and ignores numerous other neurochemical and neurophysiological effects of the respective drugs.

In the following, the efficacy of the most interesting novel anticonvulsant drugs in the respective animal models proposed in this paper will be compared with data from clinical evaluation. However, it should be critically considered that most of these novel compounds have only been tested yet in patients with partial epilepsy (most patients with complex partial seizures) resistant to conven-

tional drug therapy. The results of clinical trials of some compounds have not been published in detail in peer review journals. Some newly developed compounds, which have not been clinically evaluated yet, but show promising preclinical efficacy, will also be reviewed. Respective data are shown in Tables VI-IX. Data are somewhat incomplete because most compounds were not tested in all of the models proposed here or, at least, respective data were not published. For test drugs which are designated by codes or abbreviations, chemical structures are given in the respective table legends.

To begin with GABAimimetic drugs, the most promising drug of this category with respect to its clinical efficacy seems to be γ -vinyl GABA (vigabatrin), an irreversible inhibitor of the GABA-degrading enzyme GABA aminotransferase (GABA-T)^{77,110}. Indeed, several controlled clinical trials with vigabatrin indicate that this drug is more effective than placebo in the treatment of complex partial seizures and generalized tonic-clonic seizures^{77,110}. This stands in contrast to its low efficacy after acute single-dose administration in the MES, s.c. PTZ and kindling model (Table VI). In fact, if preclinical evaluation would have been based only on the traditional MES and PTZ tests, this drug would not have been identified as a potential antiepileptic. Experiments with seizure threshold tests, however, have shown that the drug is much more effective during prolonged administration than after acute single-dose administration, which can be explained by progressive accumulation of GABA due to the long-lasting effect on GABA degradation⁷⁶. These experiments in mice have also indicated that tolerance may develop when too high doses of vigabatrin are administered daily, which is apparently related to the development of compensatory mechanisms (e.g., reduction of GABA synthesis) within the GABA system⁷⁶. Clinically, no evidence has been found for the development of tolerance to the antiepileptic effect of vigabatrin. Interestingly, in epileptic gerbils vigabatrin proved to be much more potent than in any other animal model (Löscher and Frey, in prep.). An i.p. ED₅₀ of 50 mg/kg has been determined in this species, substantiating the high sensitivity of gerbils to manipulations of GABAer-

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gic mechanisms which has been demonstrated previously with other GABA mimetic drugs⁹³.

Similar to vigabatrin, the GABA receptor agonist *progabide*¹²⁰ is only weakly active in the traditional MES and s.c. PTZ models but shows significant anticonvulsant activity in threshold tests (Table VI). Very similar data, i.e., inefficacy in tests with (supra)maximal seizure induction but efficacy in threshold models, have been obtained with most GABA mimetic drugs tested up to date, which demonstrates that these potentially useful drugs would not have been discovered by the use of the widely employed MES and s.c. PTZ tests only⁹³. In the kindling model, progabide reduces severity and duration of the behavioural seizures and duration of the amygdalar afterdischarges but (like vigabatrin) it is not capable of totally blocking the focal seizure activity⁹⁰. In epileptic gerbils, however, progabide is highly effective with an i.p. ED₅₀ of 50 mg/kg⁹³. Clinical data on more than 1500 patients suggest that progabide might exert a therapeutic action in different types of epilepsy¹²⁰, although the therapeutic efficacy in patients with chronic complex partial seizures has been questioned. Indeed, several carefully controlled trials with rigid inclusion criteria^{21, 25, 31, 68, 143} have not confirmed earlier reports which found that the antiepileptic effect of progabide is superior to placebo. In epileptic dogs, progabide was effective in reducing the frequency of generalized tonic-clonic seizures, but severe liver toxicity developed during chronic oral treatment with daily doses of 50 mg/kg (Schwartz-Porscho, unpublished data). Progabide has been shown to exert life-threatening liver toxicity also in man³¹.

The third GABA mimetic drug which has been evaluated clinically is *THIP* (gaboxadol), like progabide a compound which stimulates postsynaptic GABA receptors, although there is evidence that THIP might only act as a partial agonist at these receptors⁶⁶. THIP is inactive in the traditional MES and s.c. PTZ tests but, like vigabatrin and progabide, increases thresholds for electroshock and PTZ-induced seizures, although the MES threshold is elevated by THIP only at high, toxic doses. (Table VI). In contrast to the 2 other GABA mimetic drugs, THIP is not able of reducing kindled seizure activity⁹⁰. The only clinical

study on this compound found the drug ineffective in 9 patients with partial epilepsy²⁵.

The preclinical data obtained with vigabatrin, progabide and THIP in models of petit mal seizures deserve some additional comment, because these data might be of clinical relevance. As shown in Table VI, these drugs are virtually inactive in the s.c. PTZ test in mice but increase the i.v. PTZ threshold for clonic seizures in this species. In rats, however, Myslobodsky et al.¹²² found that vigabatrin aggravated PTZ-induced seizures. Aggravation of seizure activity by GABA mimetic drugs has also been reported in rats with inborn petit mal seizures¹¹⁸ (see Table V). Similarly, these drugs were shown to prolong seizures in the GHB model of petit mal epilepsy in rats¹²⁸. In high doses, some GABA agonists have even been shown to induce spike-wave activity in the EEG of normal rats⁴⁴ and photosensitive baboons¹¹⁵. In fact, THIP-induced bilaterally synchronous spike-wave discharges in rats, which can be antagonized by ethosuximide but not by valproic acid⁴⁴, have been proposed as a model of petit mal epilepsy³⁰. These and other findings have led to the hypothesis that an increase in GABAergic transmission may be involved in the pathogenesis of generalized spike-wave discharges¹²⁰. Indeed, there is considerable experimental evidence that absence seizures are due to paroxysmal discharges in inhibitory pathways in the brain¹⁵. Tentatively, if mediated by GABA-related mechanisms, the anticonvulsant effect of benzodiazepines and valproic acid against petit mal seizures could then be explained by a facilitation of presynaptic GABAergic control of the intensity of GABA-mediated recurrent postsynaptic inhibitory action, whereas GABA mimetic drugs, such as progabide, which facilitate postsynaptic GABAergic action in these pathways would thereby exacerbate the seizures¹²¹. If this assumption is true, search for new anti-absence drugs by means of a model using the GABA antagonist PTZ could lead to false conclusions. However, as long as the effect of GABA mimetic drugs on human petit mal seizures has not been established, the meaning of these experimental data for absence seizures in humans remains uncertain.

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βAminetic drugs, i.e., lipophilic derivatives of guvacine and nipecotic acid such as *SKF 10030-A* and *SKF 89976-A*, which increase synaptic GABA concentrations by inhibition of GABA uptake from the synaptic cleft¹¹². As shown in Table VI, these compounds are inactive in the MES test, but show efficacy in the s.c. PTZ test. The most promising data with these drugs, however, stem from kindling experiments (Table VI and Fig. 2). In the amygdala kindling model, *SKF 10030-A* proved to be more potent in suppressing focal seizure activity than any of the clinically established antiepileptic drugs, which are active in this model^{104,151}. *SKF 89976-A* was somewhat less potent than *SKF 10030-A* but still exerted significant activity in this model. When given prior to and during amygdala stimulation in unkindled rats, *SKF 89976-A* inhibited the evolution of full kindled seizure activity¹⁵⁰. Furthermore, both compounds showed marked activity in the gerbil model of generalized tonic-clonic seizures¹⁰. Since both compounds also compared favourably with diazepam in terms of neurotoxicity^{150,151}, these data thus strongly indicate that GABA uptake inhibitors, such as *SKF 10030-A*, merit further study as new anticonvulsant drugs with a very selective mechanism of action.

Besides compounds that increase GABAergic transmission by effects on GABA receptors, GABA uptake or degradation, several substances facilitate GABA function by an effect at the BZ receptor of the GABA receptor-ionophore complex¹². The classical example is of course the 1,4-benzodiazepines, such as diazepam and clonazepam, but the same effect is also induced by the more recent 1,5-benzodiazepine, clobazam and by *β-carbolines*, which act as full or partial agonists at BZ receptors^{114,127,160,113}. As shown in Table VI, clobazam possesses the same broad spectrum of anticonvulsant activity as 1,4-benzodiazepines and is able to reduce seizure frequency in patients with complex partial seizures¹⁴⁸; however, tolerance develops to its antiepileptic effect in humans^{149,173}. Since tolerance (and dependence) seems to be an inherent disadvantage of the benzodiazepines, alternative ligands for the BZ receptor with comparable anticonvulsant action to benzodiazepines but not showing tolerance are being

sought. One class of new BZ ligands are the *β-carbolines*. Full BZ agonists of this class, such as *ZK 93423*, show the same efficacy as benzodiazepines in animal models, whereas partial agonists, such as *ZK 91296* and *ZK 95962*, seem to be ineffective in the MES and kindling models, but show activity against PTZ (Table VI). It should be noted, however, that in gerbils, *ZK 91296* was effective against both minor (petit mal like) and major (grand mal like) seizures¹⁰. The advantage of partial BZ agonists of this type is that they are much less sedative and muscle relaxant than are benzodiazepines. Preliminary clinical data indicate that *ZK 95962*, as could be expected from its effect in petit mal models, is able to reduce photosensitive absence seizures in patients¹¹⁹.

Several other drugs with diverse structures, which might act via GABAergic mechanisms, are shown in Table VII. *Gabapentin* is highly effective in blocking MES in rats and PTZ seizures in mice, but is not capable of blocking MES in mice⁷. Although the difference between mice and rats cannot be explained, the data illustrate that both species should be studied in preclinical evaluation of a test drug. *Gabapentin* has not been tested in the amygdala kindling model, but experiments with hippocampal kindled rats showed that the drug had no relevant effect on the afterdischarge threshold⁷. Preliminary clinical data, as yet not published in detail, however, suggest that *gabapentin* might possibly possess antiepileptic efficacy in human epilepsies⁷.

Stiripentol is active against both MES and PTZ seizures, but has not been tested in the kindling model (Table VII). In the alumina gel model in rhesus monkeys (in which the epileptogenic focus was activated by systemic injection of 4-deoxypyridoxine), *stiripentol* was comparable to valproate in that it delayed the onset of seizures but did not eliminate them as do other antiepileptic drugs¹⁶³. Based on very preliminary data as yet not published in detail, *stiripentol* appears to improve seizure control in some patients with focal epilepsy¹⁶³.

The *trans*-isomer of 2-en-valproic acid (2-en-VPA) is the major active metabolite of valproic acid in different species, including man^{121,124}. This compound has about the same anticonvulsant po-

TABLE VII

Anticonvulsant potency of new anticonvulsant drugs which (based on limited evidence) might act via GABAergic mechanisms

For explanation see Table IV. Abbreviations not explained in Table IV: GABA-T, GABA aminotransferase (the GABA degrading enzyme); BZ, benzodiazepine binding; n.t., not tested; 2-en-VPA, 2-en-valproic acid; CGS 9896, 2-(p-chlorophenyl)pyrazolo(4,3-c)quinolino[3,5H] one; CM 40907: 6-(2-chlorophenyl)-3-(4-hydroxy-piperidino)-pyridazine. Data are from refs. 95, 113 and unpublished experiments of our group.

Anticonvulsants drug	Potential mechanism of action	Potency in experimental models (ED ₅₀ in mg/kg i.p.)						Neurotoxicity (TD ₅₀ in mg/kg i.p.)	Antiepileptic effect in humans	
		MES (gen. tonic seizures)		i.e. PTZ (gen. clonic seizures)		Amygdala kindling in rats			Clinical efficacy	Daily dose (mg/kg p.o.)
		Mice	Rats	Mice	Rats	Focal	Gen.	ADD		
				Mice	Rats					
Clobazepam	Inhibition of GABA-T?	>2000 ^a	9.4 ^a	150 ^b		NE ^c			PS (?)	5-15
Stiripentol	Inhibition of GABA-T and uptake?		240	200					PS (?)	25-50
2-en-VPA (trans-isomer)	GABA ↑	220		225		>300	140	1	220	≥VPA n.t.
Denzinol	Increase in BZ binding?		9.7 ^a		>180 ^a	>260 ^a			58	80
CGS 9896	Partial BZ agonist?	>300 ^a	>100 ^a	15 ^a				1	PS (?)	GTCS (?)
CM 40907	Increase in BZ binding	16 ^a		77 ^a			20 ^a	1	140	n.t.

^a p.o.; ^b hippocampal kindling; ^c increase in synaptosomal GABA levels and potentiation of postsynaptic GABA function¹²⁴.

tency and spectrum of activity as valproic acid, but is somewhat more sedative than the parent drug in mice⁹⁵ (Table VII). Like valproic acid, 2-en-VPA increases presynaptic GABA levels and potentiates postsynaptic GABAergic function¹²⁴. However, 2-en-VPA has significant advantages compared to valproic acid in terms of toxicity and teratogenicity. During recent years it has become increasingly evident that therapy with valproic acid may be associated with fatal hepatotoxicity as well as serious embryotoxicity (leading to malformations, such as spina bifida) in a very small portion of patients¹²⁴. The hepatotoxic and teratogenic potential of valproic acid can also be demonstrated in respective animal models, whereas 2-en-VPA lacks the adverse effects of the parent drug in these models^{95,124}. 2-en-VPA may thus merit interest as a valuable alternative drug in antiepileptic therapy.

Denzinol is active in the MES test but inactive against PTZ seizures (Table VII). Pilot studies in a limited number of patients suggest that it may reduce the frequency of generalized tonic-clonic and

focal seizures¹⁷¹.

CGS 9896, a pyrazoloquinoline with partial BZ agonistic properties⁷¹, has not yet been tested clinically. Preclinical evaluation showed that, similar to β-carboline partial BZ agonists (Table VI), this compound is inactive against MES but blocks PTZ seizures (Table VII). In comparison to the partial BZ agonists, CM 40907, a piperidino-pyridazine derivative, has a broader spectrum of activity (Table VII), but, again, this interesting drug has not yet been tested clinically¹¹.

In Table VIII, the efficacy of novel anticonvulsant compounds with diverse mechanisms of action is shown. Oxcarbazepine, the 10-oxo-analogue of carbamazepine, possesses similar pharmacological activities as the parent drug. It exerts anticonvulsant action in the MES test in mice and rats, whereas the reported anti-PTZ effect is questionable, since in the PTZ test system used in the respective report carbamazepine was also active⁶. In cats with hippocampal stimulation, oxcarbazepine is not capable of affecting afterdischarges, but its active metabolite 10-hydroxycarbazepine is

as potent to decrease afterdischarge duration in this model as is carbamazepine⁶. As could be expected from the preclinical evaluation, in epileptic patients oxcarbazepine exerts similar antiepileptic efficacy as carbamazepine⁴. Another example for a drug which has been developed by structural modification of an antiepileptic drug is *eterobarb*¹⁷. Because of the barbiturate structure and the fact that this compound is metabolized to phenobarbital after administration, eterobarb shows comparable preclinical and clinical efficacy as phenobarbital (Table VIII).

Flunarizine is a novel compound that reduces the transmembrane influx of calcium¹⁷. However, if this effect is responsible for its anticonvulsant action is still unclear. In animal models of epilepsy, flunarizine shows activity which is very similar to that of phenytoin and carbamazepine in that MES seizures are blocked but clonic PTZ seizures are not (Table VIII). Flunarizine seems to reduce kindled seizure activity, although the efficacy against focal components of the seizures appears

to be low⁴. Based on initial clinical studies, flunarizine seemed to reduce complex partial and generalized tonic-clonic seizures¹⁷ but in a more recent randomized double-blind placebo controlled cross-over study in 30 patients with complex partial seizures, flunarizine did not decrease seizure frequency significantly¹⁶. Further carefully controlled trials are certainly required for final assessment. Somewhat surprising, it has been claimed that flunarizine's calcium entry-blocking action does not lead to negative effects on heart muscle and blood vessel tone¹⁷. Flunarizine may lead to extrapyramidal dyskinesia in some patients.

An example for a drug which, based on its pre-clinical profile, should not have been further developed is *milacemide*. This drug, which has been shown to increase brain glycine and (in substantia nigra) GABA levels¹⁸ is inactive in all standard tests shown in Table VIII. The only test in which milacemide exerted potent anticonvulsant action was the i.v. bicuculline seizure model¹⁸. In uncontrolled trials, not published in peer review jour-

TABLE VIII

Anticonvulsant potency of new anticonvulsants drugs with diverse mechanisms of action

For explanation see Table IV. Abbreviations not explained in Table IV: CBZ, carbamazepine; PB, phenobarbital; 2-APB, 2-amino-7-phosphonobutyric acid; NMDA, N-methyl-D-aspartate (a ligand for excitatory amino acid receptors); MK-801, (+)-10,11-dihydro-5-methyl-5H-dibenzo(a,d)cycloheptene-5,10 imine; n.t., not tested. Data are from refs. 4, 6, 22, 24, 113, 178 and unpublished experiments of our group.

Anticonvulsant drug	Presumed mechanism of action	Potency in experimental models (ED ₅₀ in mg/kg i.p.)						Neurotoxicity (TD ₅₀ in mg/kg i.p.)		Antiepileptic effect in humans	
		MES (gen. tonic seizures)		s.c. PTZ (gen. clonic seizures)		Amygdala kindling in rats		Mice	Rats	Clinical efficacy	Daily dose (mg/kg p.o.)
		Mice	Rats	Mice	Rats	Focal ad.	Gen. ad.				
Oxcarbazepine	Like CBZ?	20 ^a	15 ^a	23 ^a (?)				NE ^a	NE ^a	6 CBZ <CBZ	10-40
Barbituric acid	Metabolized to PB	14 ^a		47						<PB	similar to PB
Flunarizine	Calcium blocker	22 ^a			>160	?	80	?		FS (?)	30
Milacemide	Glycine ↑ ^b	>300 ^a	>300			NE	NE	1 ^a	>1000 ^a	FS (?)	30
2-APB	GABA ↑ ^b										
2-APB	Inhibition of SRF antagonists	54	8 ^a	NE		NE ^a	NE ^a		305	FS, GTCS	3-10
CBZ	NMDA antagonists	>200		270		active after i.c.v.			150	n.t.	
MK-801	NMDA antagonists?	0.35		<PB						PS (?) (tolerance)	0.01-0.03

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^a hippocampal afterdischarge in cats; the active metabolite 10-hydroxycarbamazepine is as potent as CBZ; ^b increase in brain glycine levels and GABA levels in substantia nigra; ^c cortical induced afterdischarges; ^d active against hippocampal or cortical kindled seizures.

nals, milacemide has been suggested to slightly reduce seizure frequency in patients suffering from various forms of epilepsy¹⁷⁹. Careful clinical studies are needed to show if milacemide has an antiepileptic effect in human epilepsy.

An interesting new compound is *zonisamide* which, like phenytoin and carbamazepine, seems to act via suppression of membrane hyperexcitation¹⁷⁰. This compound is active against MES and hippocampal or cortical kindled seizures, but is inactive in the PTZ and amygdala kindling model (Table VIII). Clinical evaluation of zonisamide demonstrated significant therapeutic effects against both partial and generalized tonic-clonic seizures¹⁷⁰, but the drug has recently been withdrawn because of the formation of renal calculi during long-term treatment.

2-Amino-7-phosphonoheptanoic acid (2-APH; Table VIII) represents a new class of drugs; i.e., antagonists of excitatory amino acids, which currently attract a great deal of interest in epilepsy research. 2-APH is one of the most potent compounds of a series which selectively antagonizes excitatory amino acid transmission by an effect at the *N*-methyl-D-aspartate (NMDA) receptor subtype of glutamate receptors¹¹¹. Such compounds are potent anticonvulsants when injected intracerebroventricularly, but are much less potent after

systemic administration because they penetrate the blood-brain barrier poorly^{22,24,63,111} (Table VIII). Furthermore, at doses which exert anticonvulsant action, 2-APH also exerts side-effects, such as sedation and impairment of motor coordination⁸³. Thus, these novel compounds may be less promising than initially thought. However, in this respect it is interesting to note that there is some evidence indicating that the novel anticonvulsant MK-801 (see Table VIII) also acts via antagonism of glutaminergic neurotransmission. Indeed, MK-801 has been described as a non-competitive antagonist of NMDA on the basis of binding and electrophysiological experiments¹²⁰. As shown in Table VIII, this compound is highly active in the MES test, but considerably less effective against PTZ seizures, although the ED_{50} in the latter test was still lower than that of phenobarbital¹⁷⁴. Interestingly, in contrast to drugs, such as 2-APH, MK-801 seems to be essentially free of side-effects, such as sedation and motor impairment. Preliminary clinical data indicate some antiepileptic efficacy in patients with seizures of focal origin, but efficacy was not maintained¹⁷⁴.

Various new anticonvulsant drugs with yet unsettled mechanisms of action are shown in Table IX. *Felbamate* (a propanediol dicarbamate derivative like the anxiolytic meprobamate) and *flupi-*

TABLE IX

An anticonvulsant potency of new anticonvulsant drugs for which no evidence of potential mechanism of action is available

For explanation see Table IV. Abbreviations not explained in Table IV: n.t., not tested; SR 41378, 6-(2,4-dichlorophenyl)-3-(4-hydroxy-piperidino)-pyridazine. Data are from ref. 113.

Anticonvulsant drug	Potency in experimental models (ED_{50} in mg/kg i.p.)						Neurotoxicity (TD_{50} in mg/kg i.p.)	Antiepileptic effect in humans		
	MES (gen. tonic seizures)		s.c. PTZ (gen. clonic seizures)		Amygdala kindling in rats					
	Mice	Rats	Mice	Rats	Focal setz.	Gen. setz.				
Felbamate	41	48 ^a	78	240 ^a			1550 ^a	FS (?) 30		
Flupirtine	51 ^a	47 ^a	39 ^a	26 ^a			174	FS, GAS (?) 10 (1 study)		
Lamotrigine	2.6 ^a	3.6	NE ^b		6.5 ^a			FS (?) 3		
Naftidrofural	19	2	NE				80	FS (?) 8		
SR 41378	14 ^a	...	18 ^a		10 ^a	1-	32 ^a	n.t.		

^a p.o.; ^b indicated by i.v. PTZ data; ^c cortical kindled seizures.

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ive exert a broad spectrum of anticonvulsant activity at relatively low, non-sedative doses, and preliminary clinical reports suggest that these drugs might possess antiepileptic efficacy^{124,152}. *Lamotrigine* and *nafamidone* are drugs with a more phenytoin-like action in that they are active in the MES test but inactive against PTZ (Table IX). Preliminary clinical data indicate that both drugs might reduce partial seizure frequency in human epilepsy^{9,118}. Final assessment is not yet possible. The last compound of Table IX, SR 41378, is a structural analogue of the piperidino-pyridazine derivative CM 40907, which has been shown in Table VII. As CM 40907, SR 41378 exerts a broad spectrum of activity, but has not been clinically evaluated yet¹¹.

CONCLUSIONS

A survey of preclinical data on novel anticonvulsant drugs currently undergoing clinical evaluation shows that only in few instances models of focal epilepsy, such as the kindling model, have been employed, although all these drugs are initially tested in patients with this type of epilepsy. The fact that all clinically established antiepileptic drugs of primary choice against focal epilepsy are active in the MES test should not be interpreted in that this test is a predictor of drugs effective in both generalized tonic-clonic and focal seizures. In fact, the experimental data on the GABA uptake inhibitors shown in Table IV and Fig. 2 demonstrate that significant anticonvulsant activity against focal seizures can be present in the absence of efficacy in the MES test. Although an optimal model for identifying drugs active in partial epilepsy is not yet defined, the comparative data on antiepileptic drugs recently reported^{84,94} suggest that the kindling model might be suited in this respect. An added advantage of this model is that the efficacy of a test drug against the progressive process leading to epileptogenesis can be determined. Although continued kindling in animals results in the eventual appearance of spontaneous recurrent epileptic seizures¹³², the relevance of kindling as a mechanism for epileptogenesis in human focal epilepsy cannot be assessed as yet^{24,28}. With respect to models of petit mal epilepsy, the

s.c. PTZ test is still proposed for screening purposes, although a genetic model, such as the petit mal rat model described by Vergnes et al.¹⁶¹, would be clearly preferable in the search for drugs with anti-petit mal action. In view of the high percentage of rats with this inborn syndrome and the possibility to obtain large numbers of affected animals by selective breeding, it is to be hoped that this potentially useful genetic model will replace the PTZ test in due time. However, the term petit mal and the models used in this respect need some general comment. The term petit mal is not useful for the definition of experimental or clinical seizures, because it included historically a number of rather diverse epileptic syndromes. Esquiero²⁹ established this term to describe all epileptic seizures except generalized tonic-clonic seizures. Lennox and Lennox⁴⁶ coined the term petit mal triad: pure petit mal (i.e., absence seizures), myoclonic attacks and akinetic or atonic attacks, and added a fourth member: petit mal variant or petit mal with psychomotor components. In addition, Janz⁵¹ introduced a fifth member, the impulsive petit mal. In recent years, the term absence seizure has been used widely and has been thought to give a better description¹⁴. Myoclonic seizures have been defined more clearly and include epileptic myoclonic seizures which may occur without impairment of consciousness as impulsive petit mal in juvenile myoclonic epilepsy or with impairment of consciousness as in myoclonic absence seizures. Furthermore it is recognized from a clinical perspective that absence seizures may occur in a number of epileptic syndromes, e.g., childhood absence epilepsy (pyknolepsy), juvenile absence epilepsy, juvenile myoclonic epilepsy, epilepsy with generalized tonic-clonic seizures on awakening, Lennox-Gastaut syndrome, epilepsy with myoclonic astatic seizures, and epilepsy with myoclonic absences. Myoclonic seizures may occur in benign myoclonic epilepsy in infancy, juvenile myoclonic epilepsy, epilepsy with generalized tonic-clonic seizures on awakening, Lennox-Gastaut syndrome, epilepsy with myoclonic astatic seizures, early myoclonic encephalopathy, and severe myoclonic epilepsy in infancy¹⁵. Petit mal models, such as the PTZ test and rats with spontaneous spike-wave discharges, are thought to predict drugs ef-

fective in generalized seizures of the absence type^{181,190}. However, as shown in Table V, all these models are pharmacologically characterized by high anticonvulsant efficacy of phenobarbital, benzodiazepines and (if tested) primidone, while, in terms of ED₅₀ values, valproic acid and ethosuximide are less potent, and phenytoin and carbamazepine do not exert an anticonvulsant effect. From a clinical perspective, phenobarbital and primidone are not effective against absence seizures, in fact these drugs may even increase the number of absence seizures in human epilepsy possibly through their sedative side-effects. However, both drugs are effective against myoclonic seizures, e.g., in juvenile myoclonic epilepsy, and thus it is reasonable to assume that their anti-myoclonic effect and not an anti-absence effect is relevant for their efficacy in so-called experimental petit mal seizures. Human myoclonic seizures are also sensitive to valproic acid, benzodiazepines, and ethosuximide, and are insensitive to phenytoin and carbamazepine (Table I). Thus, it appears that the petit mal models, including the PTZ test, shown in Table V, are models of myoclonic seizures rather than models of absence seizures. Like absence seizures, epileptic myoclonic seizures are associated with generalized spike-wave discharges in the EEG; in other words, the occurrence of spike-wave discharges in an animal model, such as the genetic rat model, does not necessarily imply that this is a model for absence seizures. In view of their pharmacological sensitivity, we propose that the petit mal models presently used should be designated as models for myoclonic seizures. As shown by the data on phenobarbital and primidone, efficacy against myoclonic seizures in animal models is not necessarily predictive of clinical efficacy against absence seizures. Models for absence seizures should be characterized by anticon-

vulsant efficacy of valproate and ethosuximide and inefficacy of phenobarbital, primidone, phenytoin and carbamazepine. At present, no model meets these criteria.

New animal models of epilepsy should be constantly explored for several reasons. Each new method yields information on the nature of the convulsive process and the mechanism of anticonvulsant drug action. Potential antiepileptics of novel chemical structure may not be adequately defined by animal models now in use. Finally, drug specificity for particular types of epilepsy may be evaluated better by novel methods of assay. The present proposal of preclinical systems for antiepileptic drug evaluation is similar to the simple screening battery of the NINCDS program of the NIH but adds threshold models and, most important, a highly reproducible test system for focal seizures. Furthermore, it is emphasized that not only the acute efficacy of drugs should be determined during preclinical evaluation but also the efficacy during subchronic administration of a candidate drug. The kindling model seems to be particularly suited for such studies, provided the test drug is active in this paradigm.

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